Tove Schult

Properties of acid sulfite cellulose for cellulose derivatives

NTNU Trondheim
Norges teknisk-naturvitenskapelige universitet

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PROPERTIES OF ACID SULFITE CELLULOSE FOR CELLULOSE DERIVATIVES

by

Tove Schult

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SUMMARY

The thesis comprises four parts: Existing methods for SEC analysis of cellulose have been improved. A new column material and a modified dissolution procedure have been developed. These improvements have been applied to study the effect of the pulping process on the molecular weight distribution of acid sulfite pulp. Using the SEC method made it possible to study changes in molecular weight and molecular weight distributions during derivatization to carboxymethyl cellulose. The cellulose and carboxymethyl cellulose structures were studied by NMR spectroscopy and X-ray diffraction.

Molecular characterization of cellulose by SEC has been limited by adsorption effects on the column as well as problems with incomplete dissolution and aggregation of cellulose. An alternative column material using macroporous monodisperse particles (MMP) minimized the adsorption problems compared to the commercial PLgel Mixed-B column. Improved chromatographic stability (back pressure and reproducibility) was observed in addition to high sample recovery. The MALLS data showed no aggregate present in the cellulose solution. This also indicates an improvement of the modified dissolution procedure developed for cellulose in LiCl/DMAc. Using this method made it possible to study the MWD variations of acid sulfite pulp cellulose caused by different production conditions. By increasing cooking time and temperature a shift towards lower average molecular weight and a more narrow molecular weight distribution was observed.

The main objective of the present work was to improve the SEC method of analyzing cellulose in order to study molecular changes during cellulose modifications. A comprehensive characterization of both the raw material and the product preferential by comparable methods is essential. In this work carboxymethyl cellulose has been made by three different processes and analyzed by SEC. A general decrease in average molecular weight was observed during the carboxymethylation. Otherwise, the different carboxymethylation methods acted differently. The homogeneous carboxymethylation caused a bimodal molecular weight distribution which indicates the presence of a non-random degradation.

The initial molecular structure of the cellulose raw material may affect on the degree of substitution and substitution pattern of the carboxymethyl cellulose. Therefore a structural characterization of both solid and liquid state was needed and was performed by NMR spectroscopy/X-ray diffraction and SEC-MALLS, respectively. By the R_G-M relation, the MALLS data proved that cellulose may be considered to be a random coil in 0.5% LiCl/DMAc. The NMR data showed that the crystallinity index was similar for all the acid sulfite pulp samples. NMR and X-ray results on CMC showed that the low-substituted CMC samples manufactured by the heterogeneous method seemed to retain a fraction of crystalline cellulose (both cellulose I and cellulose II). A DS above 1.0 leads

to a loss of crystalline structure. An initially high molecular weight cellulose sample retained more of its crystalline structure after the carboxymethylation.

PREFACE

This thesis is submitted in partial fulfilment of the *doktor ingeniør* degree at the Norwegian University of Science and Technology (NTNU). The work has been carried out at the Pulp and Paper Group, Department of Chemical Engineering, NTNU from 1996 to 2000 with Adjunct Professor Peder J. Kleppe and Associate Professor Størker T. Moe as supervisors.

The work in this study is a part of the project "Development of new high alpha cellulose pulps". The doctoral study was funded by Borregaard ChemCell and the Norwegian Research Council (NFR).

This thesis includes the following papers as journal articles referred to as **Paper I-II** in the text:

Paper I:
Schult, T., Moe, S.T., Hjerde, T. and Christensen, B.E. Size exclusion chromatography of cellulose dissolved in LiCl/DMAc using macroporous, monodisperse poly(styrene-*co*-divinylbenzene) particles. Accepted for publication in *Journal of Liquid Chromatography* (2000)

Paper II:
Schult, T., Hjerde, T., Optun, O.I., Kleppe, P.J. and Moe, S.T. Characterization of cellulose by SEC-MALLS. Submitted for publication in *Macromolecules* (2000)

Results related to this thesis but not included in the papers above have also been presented at the following international conferences:

Schult T., Moe S.T. and Kleppe P. J. Changes in cellulosic structure during carboxymethylation studied by NMR spectroscopy and X-ray scattering, *Proceedings 10th ISWPC*, III-180, Yokohama, 1999

Schult T., Christensen B.E. and Moe S.T. Size exclusion chromatography of cellulose: A novel application of macroporous monodisperse particles, *19th International Carbohydrate Symposium*, Oral presentation, DO 021, San Diego, 1998

Schult T. and Moe S.T. Viscosity loss and molecular weight degradation during etherification of high-molecular weight cellulose, *Proceedings 9th ISWPC*, 99-1, Montreal, 1997

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ABBREVIATION LIST

a	Mark-Houwink-Sakurada exponent
AGU	Anhydroglucose unit
A_2	Second viral coefficient
α	Exponent in the R_G-M relation
B	Applied magnetic field
B_0	Magnetic field
c	Concentration by weight
CED	Cupriethylenediamine
CLC	Cotton linter cellulose
CMC	Carboxymethyl cellulose
CP-MAS	Cross polarization magic angle spinning
C1-C4	Samples of cotton linter cellulose
DCM	Dichloromethane
DMAc	*N,N*-dimethylacetamide
DMSO	Dimethyl sulfoxide
DOC	Dissolved organic carbon
DP	Degree of polymerization
DS	Degree of substitution
DTPA	Diethylene triamine pentaacetic acid
D_2O	Deuterium oxide
D1-D8	Samples of acid sulfite pulps
$(dn/dc)_\mu$	Refractive index increment
E	Energy
EDTA	Ethylene diamine tetraacetic acid
γ	Magnetogyric ratio
$\dot{\gamma}$	Shear rate
h	Planck's constant
HMDSO	Hexamethyldisiloxane
HR-MAS	High resolution magic angle spinning
HV	High viscosity
$[\eta]$	Intrinsic viscosity
η_{red}	Reduced viscosity
η_{rel}	Relative viscosity
η_{sp}	Specific viscosity
I	Momentum of inertia
I	Spin number
k	Boltzman's constant
K^*	Optical parameter equal to $4\pi^2n^2(dn/dc)^2/(\lambda_o{}^4N_A)$
L	Lumen
LALLS	Low angle laser light scattering

LiCl	Lithium chloride
LS	Light scattering
LV	Low viscosity
λ_0	Wavelength of the scattered light in vacuum
m	Mass
m_I	Magnetic quantum number
M	Middle lamella
M	Molecular weight
M_0	Molecular weight of one AGU
$\overline{M}_n$	Number average molecular weight
$\overline{M}_v$	Viscosity average molecular weight
$\overline{M}_w$	Weight average molecular weight
$\overline{M}_z$	z-average molecular weight
MALLS	Multi angle laser light scattering
MAS	Magic angle spinning
MCA	Monochloroacetic acid
MCC	Micro crystalline cellulose
MeOH	Methanol
Me_2SO	Dimethyl sulfoxide
Me_4U	1,1,3,3-tetramethylurea
MHS	Mark-Houwink-Sakurada
MMP	Macroporous monodisperse particle
MV	Medium viscosity
MW	Molecular weight
MWD	Molecular weight distribution
μ	Magnetic dipole moment
n	solvent refractive index
NaOH	Sodium hydroxide
NMR	Nuclear magnetic resonance
NOESY	Two-dimensional nuclear Overhauser spectroscopy
N_A	Avogadro's number
n	Number of moles
N_α	Number of nuclei populated in the higher energy level
N_β	Number of nuclei populated in the lower energy level
ν	Frequency
ν_0	Larmor frequency
P	Primary wall
PS1-PS7	Samples of polystyrene standards
Pi	Polydispersity index
r	Distance
R_G	Radius of gyration
RALLS	Right angle laser light scattering
RI	Refractive index
$R(Q)$	The excess intensity of scattered light at an angle Q
SCAN	Scandinavian pulp, paper and board standardized test

SEC	Size exclusion chromatography
SLS	Static light scattering
S_1	Outer secondary wall
S_2	Middle secondary wall
S_3	Inner secondary wall
t_s	Time flow of solution
t_l	Time flow of solvent
T	Temperature
TFA	Trifluoroacetic acid
TSP	3-(trimethylsilyl)-propionic acid-2,2,3,3-d_4
UV	Ultraviolet absorption detector
V	Voltage
V_r	Retention volume
V_s	Selective permeation volume
V_t	Total permeation volume
V_0	Void volume
Visco	On-line viscometry

CHAPTER

1

INTRODUCTION

1.1 Motivation

The sulfite process is along with the kraft process the main method for chemical pulp production. Its main features were elucidated in the 1870's by C.D. Ekman. In the early years the sulfite process had the advantage of yielding the brightest unbleached chemical pulp, and the most easily bleached one. However, the sulfite process suffered from two distinct disadvantages: (1) only a limited number of wood species could be pulped and (2) the pulps produced were distinctly weaker than those produced by the kraft process. Today the sulfite process supplies only approximately 6% of the total paper-grade chemical pulp production world wide. The sulfite process continues to be used mainly for specific end products such as speciality pulp, high purity cellulose for chemical applications, dissolving pulp and pulp for tissue, glassine and greaseproof paper. In Norway about 150 kton of sulfite pulps are produced annually, mainly by Borregaard Industries Ltd.

Efforts to improve the sulfite process are continuously being carried out to produce pulp with pre-determined properties. Much research have been focused on obtaining a high alpha-cellulose content, the degradation and loss of carbohydrates and what implication these factors have on the final pulp properties. To study the process, it is important to characterize the pulp and other organic compounds during the cooking and bleaching. In addition, the final pulp has to be characterized in order to 1) control the process and achieve a satisfactory quality 2) evaluate the influence of process changes. It is therefore necessary to improve known methods for chemical characterization and for the application of new analytical techniques. In high-alpha cellulose products, only minor amounts of hemicellulose, lignin, resins and inorganics will be present. The chemical structure and the molecular weight of cellulose are therefore important parameters in relation to the physical and chemical properties of the pulp.

Chemical pulp is a heterogeneous product in several respects and this complicates the characterization of the chemical composition and the physical properties. Bulk properties such as viscosity, alkali solubility and kappa number are frequently used to describe the

extent of carbohydrate degradation and delignification. A more detailed information of the pulp is achieved through a separation of the polymer and a subsequent measurement of the molecular weight distribution (MWD). Size exclusion chromatography (SEC) is at present the most widely used method for measuring MWD. The main obstacle for a chromatographic separation of cellulose will be its limited solubility in common solvents and the requirement that the solvent used has to be compatible with the column material.

The molecular weight characterization of cellulose by SEC allows a study of the influence of different raw materials and carboxymethylation methods on the CMC product. Generally, to understand the macroscopic behaviour of carboxymethyl cellulose, such as a water binder, thickener and an emulsion stabilizer, a precise knowledge of both the chemical structure and molecular conformation is necessary. This is possible by using the SEC method for both cellulose and CMC.

1.2 Objectives of the work

The objectives of the work have been:

1. to improve existing methods and develop new methods for determining the molecular structure of high alpha-cellulose pulps and CMC products.
2. to apply these analytical methods for investigating new variation in manufacturing conditions influences the MW, the MWD and structure of high alpha-sulfite pulps and CMC.
3. to use NMR and X-ray for structural characterization of the cellulosic materials and CMC in solid state and MALLS for determining the structure of cellulose in solution (LiCl/DMAc).

CHAPTER 2

BACKGROUND

2.1 Cellulose

2.1.1 The structure of cellulose

Cellulose is a linear homopolymer composed of β-D-glucopyranose units which are linked together by (1-4)-glycosidic bonds (Figure 2.1). Each anhydroglucose unit (AGU) possesses hydroxy groups at C-2, C-3 and C-6 positions. These are capable of undergoing typical reactions known for primary and secondary alcohols. The cellulose chain has a reducing (aldehyde hydrate group) and a non-reducing (alcoholic) hydroxy end group which show different behaviour. Cellulose samples consist of a mixture of molecules with varying chain length and are thus polydisperse. The maximum degree of polymerization (DP) of native wood cellulose is assumed to be 10000 [1].

The generally assumed conformation of AGU in the cellulose chain is the $^4C_1$ chair conformation. The free hydroxyl groups are positioned in the ring plane (equatorial), while the hydrogen atoms are in a vertical position (axial). The bridging and ring oxygen atoms are predominantly involved in forming the hydrogen bonds and in degradation reactions [2]. The β–link requires that the plane of the pyranose ring of every second glucose unit along the molecular chain is turned around the C(1)-C(4) axis by 180° with respect to the glucose units lying in between. This means that cellulose has a cellobiose repeating unit for each 1.3 nm.

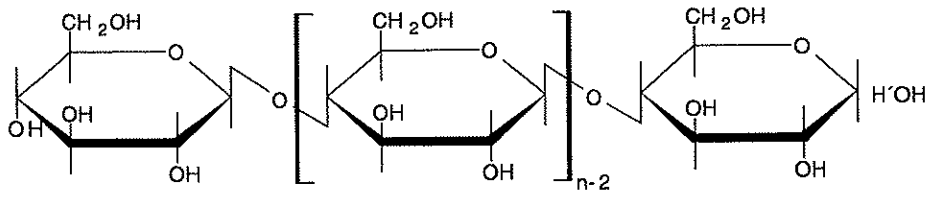

Figure 2.1 The cellulose chain.

Deviations from the described structure of cellulose is observed in bleached cotton and wood pulp. The presence of carboxylic acid groups and some carbonyl groups influences the conformation and properties of the cellulose molecule.

2.1.2 Hydrogen bonding

Besides chemical reactions, the three hydroxyls in each AGU have the possibility of interacting with each other forming secondary bonds. There are two types of hydrogen bonds, intramolecular (in the same molecule) and intermolecular (between neighboring molecules).

Native crystalline cellulose has intramolecular hydrogen bonds between O-3-H and O-5´, and between O-2-H and O-6´ [3]. These hydrogen bonds are responsible for the considerable stiffness of the cellulose chain. They also stabilize the two-fold helix conformation of crystalline cellulose which is reflected in the high viscosity of cellulose solutions, high tendency to crystallize and the ability to form fibrillar strands.

The presence of intermolecular hydrogen bonds is the predominant factor responsible for interchain cohesion and is favoured by the high regularity of the hydrogen bond and by the involvement of all three hydroxyl groups in the network. Intermolecular hydrogen bonds are formed between O-6-H of one chain and O-3 of another chain [4].

2.1.3 The supramolecular structure

The order of the macromolecules in cellulose fiber is not uniform throughout the whole structure, but is divided in regions of low order (amorphous) as well as very high crystalline order [5]. A two-phase model with crystalline and amorphous regions (fringed-fringle model [3]) is generally accepted today and important for understanding the heterogeneous cellulose reactions. The degree of crystallinity of different cellulose samples covers a wide range and depends on origin and pretreatment of the sample (Table 4.7).

Bundles of cellulose molecules are stacked together in microfibrils where highly ordered and less ordered regions are alternating. Microfibrils builds up fibrils and further cellulose fibers. Because of this fibrous structure and strong hydrogen bonds, cellulose has a high tensile strength and is insoluble in common solvents.

The ordered cellulose may exist in more than one crystalline form (polymorphs) [6, 7]. Cellulose I can be transformed to cellulose II by swelling the cellulose in strong alkali solution (mercerization). Minimum energy considerations favour a parallel arrangement of cellulose I and an antiparallel of cellulose II [8]. However, this total rearrangement of the cellulose structure is difficult to imagine and no proper explanation and mechanism has been proposed.

The hydrogen bond system of cellulose II appears to be more complicated than that of cellulose I and results in a higher intermolecular crosslinking density. In the unit cell structure of cellulose II, all the hydroxyls are positioned favourably for the formation of intermolecular and intramolecular hydrogen bonds.

2.2 Cellulose isolation from wood

Dissolving and derivative pulps are the raw material for the production of many different end-products. Corresponding to the multiple reaction routes, different properties concerning their chemical composition, as well as their average molecular weight and molecular weight distribution may be obtained. These properties are considerably influenced by the raw material used, the pulping process and purification treatments. For dissolving pulp production from wood, only the acid sulfite process and the prehydrolysis kraft process are of major practical importance [9].

As raw material hardwoods and softwoods are used, both with specific advantages and disadvantages. With regard to the suitability of different wood species for the dissolving and derivative pulp production the chemical composition, the percentage of the cell types and their morphological structure is of importance.

2.2.1 Wood fibre structure

Wood is composed of elongated cells, which are mostly oriented in the longitudinal direction of the stem. They are connected with each other through openings, called pits. The softwood cells (tracheids and ray parenchyma cells) are varying in shape and function. According to their functions the cells should [4]: 1) provide the necessary mechanical strength to the tree, 2) perform the function of liquid transport and 3) storage of the reserve food supplies. Nutrition and water are exchanged through pores in the living cell wall.

The main molecular components in wood are cellulose, hemicellulose and lignin. The amount and composition of these components vary between different cell types. The same constituents are distributed differently in the fiber cell wall layers around the central cavity (Figure 2.3), which is called lumen. A schematic description of the cell wall is shown in Figure 2.2.

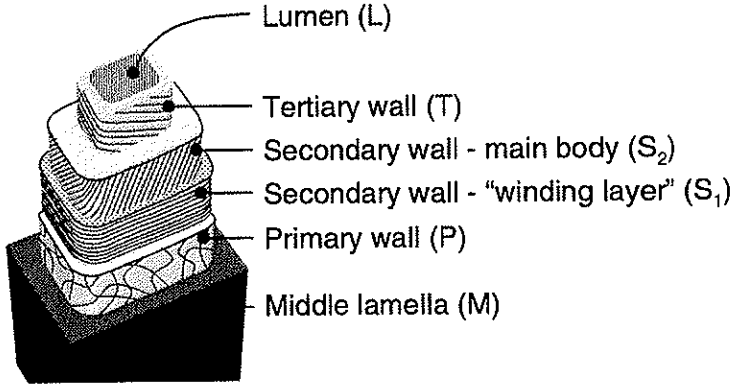

Figure 2.2 Illustration of fiber structure showing the middle lamella (M), primary wall (P), the winding (S_1) and main body (S_2) layers of secondary wall, tertiary wall (T) and the lumen (L). Adapted from Moe [10].

Generally, hemicellulose and lignin form a matrix around the microfibrils of cellulose. These are arranged in an irregular way in the primary wall (P), but have distinct directions in each secondary wall layer.

Norway spruce (*Picea abies*) consists of approximately 42% cellulose, 29% hemicellulose and 28% lignin. The remaining 1% consists of extractives and inorganic material. In spruce the S_2 layer is richest in cellulose whereas the relative amount of hemicellulose increases toward the lumen (Figure 2.3). The main part of lignin is located in the thickest secondary wall layer (S_2). However, the highest concentration of lignin is found in the middle lamella (M).

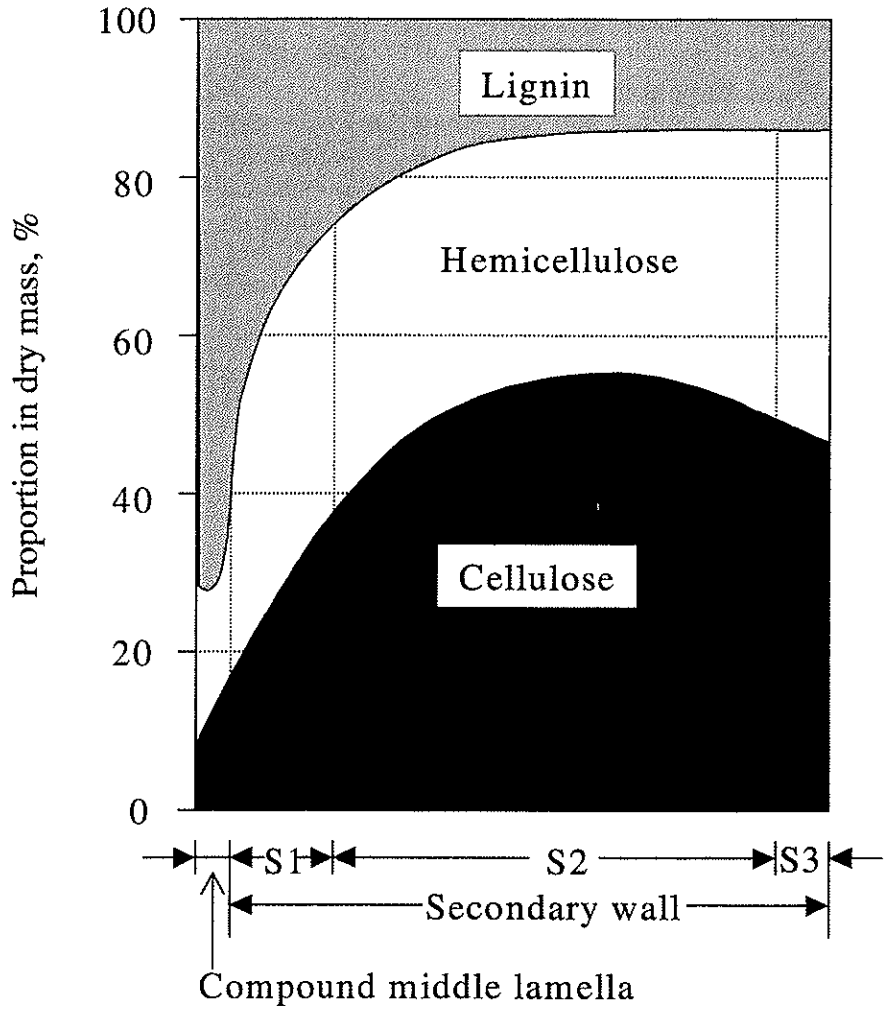

Figure 2.3 Distribution of cellulose, hemicellulose and lignin within the cell wall in conifers (redrawn from Panshin and de Zeeuw [11]).

2.3 Chemical modification of cellulose

Cellulose can be modified by chemical reactions (esterification, etherification and oxidation) in several ways [12, 13]. Good film-forming properties of the cellulose derivatives can be achieved by the introduction of hydrophobic alkyl groups, whereas hydroxyalkylation results in good solubility [14].

The chemical and physical properties of the derivatives are influenced by the types of substituents, the degree of substitution (DS) [15], the uniformity of substitution, the degree of polymerization (DP) and the distribution of molecular weight (MWD) [16].

The DS is defined as the average number of hydroxyl groups substituted in one glucose unit. Physical properties such as solubility and swelling are strongly affected by changing the DS (maximum value of 3). Introduction of hydrophobic substituents makes cellulose soluble in alkaline solutions or water because of the destruction of crystalline regions, which are formed by inter- and intra hydrogen bonding among hydroxyl groups. The uniformity of substitution is governed by the relative reactivity of the hydroxyl groups. The derivatization of cellulose is generally conducted under heterogeneous conditions, while all hydroxyl groups along a cellulose chain are not equally accessible to reagents.

2.3.1 Carboxymethyl cellulose (CMC)

Carboxymethyl cellulose represents the commercially most important cellulose ether with an annual production of about 300,000 tons worldwide. The recent research on CMC has been directed mainly toward process optimization and rationalization. Scientific progress has been achieved in the chemical modification, analytical characterization [17, 18] and in the understanding of the anionic polyelectrolyte nature [19], especially in aqueous solution [20], of CMC.

CMC is commercially prepared from alkali cellulose with sodium monochloroacetate or monochloroacetic acid as reagent. For the preparation of cellulose ethers two principal reaction types are being used: 1) reactions with alkali consumption and 2) reactions without alkali consumption. The preparation of carboxymethyl cellulose by the reaction of alkali-cellulose with monochloroacetic acid proceeds according to the alkali consumption reaction mechanism (Figure 2.4). In the course of these reactions sodium hydroxide is consumed and has to be present in sufficient supply. The reaction is irreversible and results in a rate controlled substituent distribution.

CMC is manufactured in the DS range from 0.4 to about 1.4 and the DP varies from 200 to 3000. The water solubility of CMC is increased by increasing the DS. At DS values of 0.6 - 0.8 good water solubility is obtained, whereas preparations with a DS of 0.05 - 0.25 are soluble only in alkali. Because of the carboxyl groups, CMC is a polyelectrolyte. Its pKa value varies from about 4 to 5, depending on the DS. An increasing polyelectrolyte concentration may leads to increased aggregation.

Viscosities of Na-CMC solutions become lower with increasing temperature [21]. Under normal conditions, the effect of temperature on viscosity is reversible. However, long

periods of heating at high temperatures will tend to depolymerize Na-CMC and reduce its viscosity. Na-CMC solutions exhibit their maximum viscosity and best stability at pH 7-9. Below pH ~ 3, precipitation of the free acid form of CMC may occur. CMC may undergo acid-catalyzed hydrolysis in acidic solutions, resulting in permanent loss of viscosity. Above pH 10 only a slight decrease in viscosity is observed.

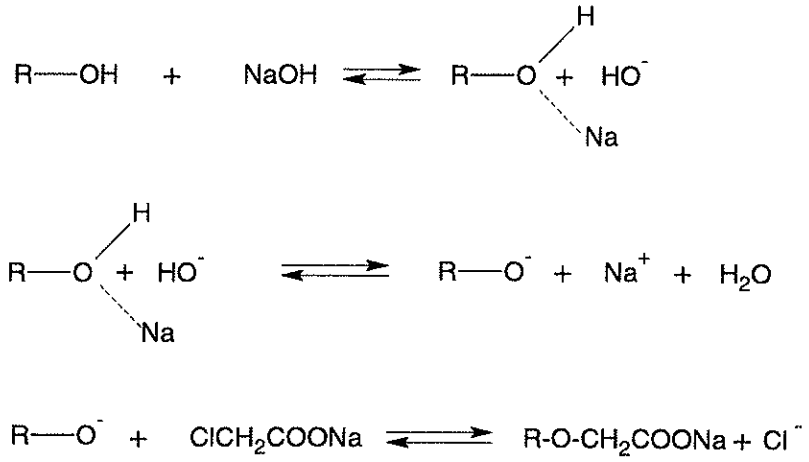

Figure 2.4 Mechanism for preparation of carboxymethyl cellulose [3]

The basic properties that enhance the commercial value of CMC are its abilities to thicken water and suspend solids in aqueous media. These properties have been utilized in widely different applications.

1. Detergents: Na-CMC can improve the detergency. The soil-suspending properties of detergents are caused by electrostatic repulsion between the negatively charged dirt particles and the negativity induced by the carboxymethyl group. Na-CMC can replace much of the fatty acid soaps in washing compounds on both an economic and a performance basis.
2. Food: Chronic and acute toxicity investigations have shown that Na-CMC is physiologically inert. As a binder and thickener, cellulose gum functions to control consistency in jellies, pie fillings, cheese spreads, puddings etc. Its water binding action retards undesirable crystal growth in ice cream and sugar products (syrup and dessert toppings). Aqueous systems of fats, flavors and fruit juices can be converted to dry, stable product by dehydration in the presence of cellulose gum. As a carrier for vitamin C, Na-CMC is useful in the preservation of fruit.
3. Pharmaceuticals and cosmetics: Na-CMC, which is insoluble in stomach acid but soluble in alkaline intestinal fluids, has replaced other water-soluble gums as an enteric coating for powders and tablets. It is a mild but effective bulk laxative because of its hydrophilic nature. Its suspending action makes Na-CMC valuable for aqueous system containing calamine and antibiotics etc.

4. Drilling fluids: In the oil industry, drilling muds are used to lubricate and cool the bit and to carry cuttings away from the bore hole. These fluids are primarily aqueous dispersions of clay, bentonite and weighting agents. Water-loss inhibitors are used in these systems to facilitate viscosity control and to minimize water-loss into porous strata. The deflocculating characteristics and thickening action of Na-CMC make it an efficient water-loss agent.

The properties of CMC is also utilized in other applications like paper, textile, protective coatings, agriculture, ceramics, etc.

2.3.2 Accessibility and reactivity

As previously described each AGU within the cellulose chain has three reactive hydroxyl groups. One prerequisite for etherification reactions of cellulose is the ionization of the hydroxyl groups. Due to the inductive effects of neighboring substituents, the acidity and the tendency for dissociation increases according to the series: OH-6 < OH-3 < OH-2. It can therefore be understood why OH-2 generally is most readily etherified compared to the other hydroxyls. After substitution of OH-2 the acidity of OH-3 is usually increased which results in higher reactivity.

Another important factor in the chemical reaction of cellulose concerns the accessibility of the hydroxy groups in the AGU´s for the reactants. Accessibility depends on the available inner surface, supramolecular order and fibrillar architecture in the cellulose molecule. Only AGU´s situated at the inner surface of the pore and void system are accessible for sorption of inert gases. The hydroxyl groups located in the amorphous regions are highly accessible and react readily by etherification, esterification and oxidation reactions, whereas those in crystalline regions with close packing and strong interchain bonding may be completely inaccessible. Preswelling of the cellulose is therefore necessary (in both etherification and esterification) to improve the amount of accessible hydroxyl groups. The swelling agent destroys the crystallinity and causes a substantial increase in reactivity. Highly ordered crystalline regions of cellulose may be accessible by aqueous NaOH of suitable concentration.

Because of the more dense structure and greater involvement of the hydroxyls in the hydrogen bonding of cellulose II, so-called hornification occurs more readily in this structure when the cellulose is dried. This may be the reason for the often observed lower reactivity by reduction of the accessible interfibrillar surface of the cellulose II structure [3].

2.3.3 Swelling and dissolution properties

For limited swelling, the gross structure of cellulose is largely maintained despite an increase in sample volume. This volume change is due to the uptake of swelling agent and significant changes of the physical properties. Dissolution means a transition from a two-phase system to a one-phase system and that the original supramolecular structure of a sample is destroyed.

The extent of swelling depends on the solvent as well as on the nature of the cellulose sample. For native cellulose with a fibrous structure more drastic changes occurs

depending on whether the swelling is interfibrillar or intrafibrillar. Swelling can occur in the easily accessible amorphous regions of cellulose (intercrystalline swelling) with only minor effect on the crystalline regions.

Cellulose is highly hydrophilic due to interaction of its hydroxy groups with water molecules, but is hindered from being dissolved in water by its ordered supramolecular structure. Cellulose swells in electrolyte solutions because of the penetration of hydrated ions which require more space than the water molecules [2]. Intracrystalline swelling can be accomplished by concentrated solutions of strong bases or acids and also for some salts. However, alkali is capable of dissolving fragments of low molecular weight cellulose.

A *limited* subtype is achieved when the swelling agent is combined with the ordered cellulose in a certain stoichiometric proportion. This does not completely destroy the interfibrillar bonding. For the *unlimited* subtype, the swelling agent forms complexes with cellulose resulting in breakage of the adjacent bonds and separation of the chains so that gradual dissolution occurs.

2.3.4 LiCl/DMAc as a solvent for cellulose

The solvent is generally described as a complex between lithium and DMAc with a free chloride anion $[Li \cdot xDMAc]^+Cl^-$ where $x \sim 4$ [22]. The dissolution of cellulose is thought to proceed by exchange of one molecule of DMAc by one hydroxyl group of cellulose [23]. A number of structural models of the solvent and for the solvent-cellulose complex have been proposed and reviewed [24, 25]. Although much is understood about interaction between LiCl/DMAc and carbohydrate molecules [26, 27], a great deal remains to be known for the mechanism of dissolution.

The sample preparation for SEC analysis of cellulose requires activation either in water [28] or by refluxing with DMAc [29]. McCormick [24, 30] developed a technique involving swelling the cellulose in water overnight followed by a solvent exchange subsequently with methanol and DMAc before adding cellulose to 9% DMAc/LiCl. The swollen cellulose went into solution at room temperature and at concentration up to 15%. Ekmanis [28] developed another method and proposed an activation of the cellulose by using hot vapours of DMAc at 150°C, cooled to about 100°C before the LiCl was added. The suspension was stirred for several hours until the cellulose was dissolved.

2.3.5 Degradation of cellulose

Degradation of cellulose has important consequences for the chemical processing of cellulose. In the preparation or manufacture of cellulose esters and ethers, high solution viscosity is the decisive quality criteria. The chain degradation of a high DP starting material should therefore be limited. This is achieved by minimizing the activity of chemical degradation predominantly via minimizing the oxygen content.

The three dominating chemical degradation mechanisms of polysaccharides are described below.

1. Acidic hydrolysis

Acidic hydrolysis is a water splitting reaction which in the case of polysaccharides results in the breaking of glycosidic bonds and decreases the DP. The mechanism of acid hydrolysis of glycosidic linkages is shown in Figure 2.5. The acid hydrolysis proceeds in three stages: 1) the rapid protonation of the glycosidic oxygen atom, 2) a slow transfer of the positive charge to C-1 leading to the acyclic carbonium cation and the simultaneous split of the glycosidic linkage, and 3) the fast addition of water to the carbonium ion.

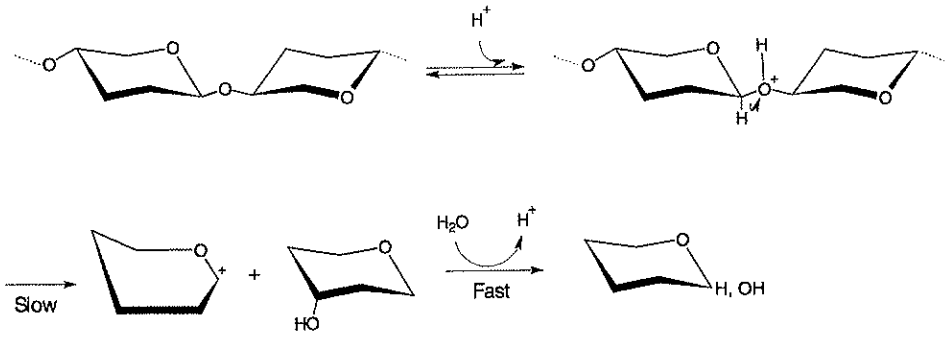

Figure 2.5 Mechanism for acid hydrolysis of glycosidic linkages [31]

All polysaccharides do not hydrolyse at the same rate. Table 2.1 shows the hydrolysis rates of different glycosides. Several factors are influencing the acid hydrolysis of polysaccharides: 1) Type of glycosidic bond: β-glycosidic bonds are more stable than α. Hexopyranosides are more stable than pentopyranosides (cellobiose vs. xylobiose), and furanosides form relatively weak glycosidic bonds. 2) Location of the glycosidic bond within the polysaccharide chain and the length of the polysaccharide. The rate is higher at the end of a polysaccharide due to increased flexibility. 3) Inductive effects (carboxyl group at C-5). Uronic acids have strong glycosidic bonds due to the presence of the carboxyl group.

2. Reactions in aqueous alkali solution

The carbohydrate chemistry in alkali involves a number of reactions. The alkaline swelling causes physical changes in the fiber wall with solvation of the hydroxyl groups. Carbohydrate molecules which are easily accessible and of low molecular weight are separated from their adjacent molecules and physically dissolved in the alkali. The dissolved material is further decomposed to a larger extent in the solution.

The peeling reaction starts at about 100 °C and is responsible for most of the carbohydrate degradation. A prerequisite for peeling reaction to occur is an initial rearrangement of a reducing end group to a 2-keto intermediate. One glucose unit is removed at a time by a further β-alkoxy elimination reaction. A competing stop reaction is taking place by conversion of the reducing end to a stable carboxylic acid group through a β-hydroxy elimination reaction.The peeling and stopping reactions are in competition with one another but the peeling reaction is faster. The product of both the

stopping and peeling reactions are saccharinic acids. About 50-80 units is cleaved off before the stopping reaction occurs.

Table 2.1: Relative acid hydrolysis rates [32]

Methyl-β-D-pyranoside	Rate	β-D-(1-4)-linked Disaccharides	Rate	Cellulose Oligosaccharides	Rate
Glucose	1.0	Cellobiose	1.5	Cellobiose	3.5
Galactose	3.0	Glucopyranosyl-Mannose	1.5	Cellotriose	2.1
Mannose	4.8	Pseudocello-biuronic Acid	1.5	Cellotetraose	1.7
Xylose	5.8	Cellobiuronic Acid	0.05	Cellopentaose	1.5
		Mannobiose	2.7	Cellohexaose	1.4
		Lactose	3.6	Cellulose	1.0
		Xylobiose	11		

The cleavage of glycosidic bonds by alkali (alkaline hydrolysis) is usually extremely slow in comparison with acid-catalyzed hydrolysis [4, 33]. However, the rate of alkaline hydrolysis becomes more pronounced at temperatures above 150 °C. The alkaline hydrolysis creates new reducing ends which are subjected to further peeling reactions. The probable mechanism of alkaline hydrolysis is outlined in Figure 2.6.

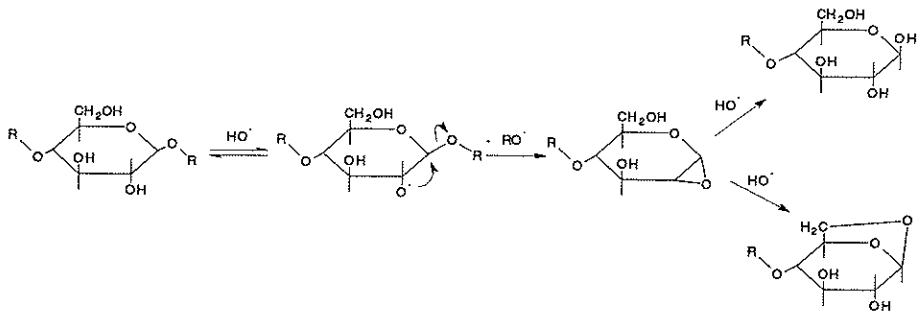

Figure 2.6 Mechanism for alkaline hydrolysis of glycosidic linkages (redrawn from Fengel [34]).

3. Oxidative degradation

Oxidative degradation of cellulose can proceed via various routes comprising numerous parallel and consecutive steps of reactions. Oxidative degradation usually starts with the conversion of single anhydroglucose units (AGU) to a fairly unstable derivative followed by ring opening and/or chain cleavage. The functional groups attacked by oxidation are the aldehyde end groups and the hydroxyl groups lead to the formation of aldehyde,

ketone and carboxyl groups. Radical as well as ionic reaction steps may promote the oxidative degradation.

Cellulose exhibits a high stability to atmospheric oxygen under neutral and acidic conditions. Elemental oxygen at elevated temperature is now widely used in bleaching of pulp suspensions (buffered to pH>9) in order to remove lignin. Ozone (O_3) is a very strong oxidizing agent which is employed as a bleaching agent for removal of lignin at a pH of about 2.5. Ozone causes only a moderate reduction of chain length of the cellulose molecule. The oxidation is not selective and some of the oxidation agent is consumed by the carbohydrates. This introduces carboxyl and carbonyl groups on the cellulose molecule. By a following alkali extraction or bleaching step with alkali this may cause a considerable loss in molecular weight by a set of auto-oxidation reactions at relatively low temperatures or by β-elimination reaction on monomers carrying carboxyl groups.

Generally, carboxyl groups in the polysaccharide molecule will stabilize the molecule toward hydrolysis. Carbonyl groups, on the other hand, enhance the rate of hydrolysis in both acidic and alkaline medium, and by introduction of such a group, a β-elimination may cause chain degradation inside the molecule.

2.4 Principles and theory of analytical techniques

2.4.1 Size exclusion chromatography (SEC)

SEC is a method for separating molecules based on their molecular size by using stationary phases with pore sizes capable of discriminating among the analytes in a sample. These porous particles may be made of polystyrene-divinylbenzene. The primary application of SEC has been in the analysis of polymers, and it is commonly used to obtain molecular weight distributions of polymers.

Resolution for SEC is determined by retention as a function of molecular size and column efficiency or bandwidth. Retention depends on molecular size and column-packing pore diameter. This relationship is best expressed in terms of a calibration plot of log (molecular weight) vs. retention time or retention volume. A sample containing a variety of molecular sizes is introduced to a SEC column with pores of about the same size as the analytes. The largest molecules will be unable to penetrate the pores and will co-elute in the same volume, void volume, V_0. Slightly smaller molecules will be able to penetrate some of the pores and will be slightly retained (Figure 2.7). The separation continuous until a critical point is reached were the molecules of given size can penetrate through all the pores. All molecules of that size and smaller will then elute together and represents the limited power of column separation. This is called the total (permeation) volume, V_t. The selective permeation volume, V_s, which is available for separation is therefore defined as

$$V_s = V_t - V_0 \qquad (2.1)$$

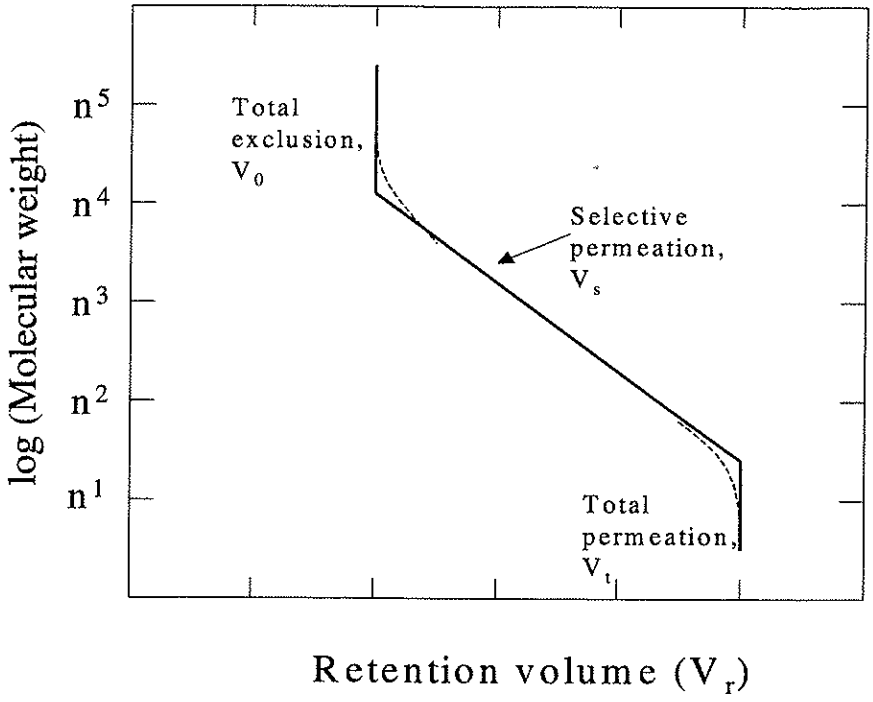

Figure 2.7 Illustration of permeation and exclusion limits in SEC [35].

The molecular weight of the sample decreases logarithmically with increasing elution volume. Molecular weights can be determined by RI and UV with calibration plots of monodisperse standards with known molecular weight. However, only light scattering provides the absolute molecular weight, molecular weight distribution and size of macromolecules in solution.

2.4.2 Light scattering theory

Light that is scattered at the same wavelength as the incoming light is called Raylight scattering. From Raylight scattering one can determine the polymer's radius (R_z) and molecular weight (M_w). This static light scattering (SLS) method is classified as an absolute method for determining molecular weight.

Based on Zimm´s formalism, the Rayleigh-Debye-Gans light scattering model for dilute polymer solutions can be expressed as

$$\frac{K^*}{R(\Theta)} = \frac{1}{M_w \cdot P(\Theta)} + 2 \cdot A_2 \cdot c \qquad (2.2)$$

where $R(\Theta)$ is the excess intensity of scattered light at an angle Θ, c is the sample concentration, M_w is the weight-average molecular weight (molar mass), A_2 is the second viral coefficient, K^* is an optical parameter equal to $4\pi^2 n^2 (dn/dc)^2/(\lambda_o{}^4 N_A)$, n is the

solvent refractive index and *dn/dc* is the refractive index increment, N_A is Avogadro's number and λ_0 is the wavelength of the scattered light in vacuum.

The function P(Θ) describes the angular dependence of scattered light and the expansion of 1/P(Θ) to first order gives:

$$\frac{1}{P(\Theta)} = 1 + \frac{16\pi^2}{3\lambda^2} \cdot \langle R_G^2 \rangle \cdot \sin^2(\Theta/2) + f_4 \cdot \sin^4(\Theta/2) + \ldots \qquad (2.3)$$

At low angles the angular dependence of light scattering depends only on the mean square radius $\langle R_G^2 \rangle$ and is independent of molecular conformation or branching. A plot of $K^*c/R(\Theta)$ vs. $\sin^2(\Theta/2)$ (Zimm plot) yields a curve whose intercept gives $\overline{M}_w$ and whose slope at low angles gives $\langle R_G^2 \rangle$.

2.4.3 Viscometric measurements

Staudinger and Heuer recognized that the viscosity of a polymer solution is closely related to the length of the chain-like macromolecules. Staudinger's original proposal was that at low concentrations there exists the relationship

$$\eta_{sp} = K_m \cdot M \cdot c_{gm} \qquad (2.4)$$

where c_{gm} = concentration in moles of anhydroglucose units per litre. This relationship is used most frequently in the form

$$[\eta] = K_m \cdot M \qquad (2.5)$$

Kuhn, Mark and Houwink recognized in the late 1930's that Staudinger's relationship was only one of the two border cases of a more general relation

$$[\eta] = K_m \cdot M^a \qquad (2.6)$$

However, today most investigators accept the simple Staudinger equation valid for stiff coil polymer chains. It can be applied without substantial error. The *a*-value reported for cellulose derivatives, however, deviates in most cases from 1.0 and ranges from 0.75-1.0.

Viscosity determinations of cellulose can be performed on solutions in various solvents, such as cuprammonium, cupriethylene diamine and quarternary organic bases. In working with cellulose derivatives it is more easy to operate with fully substituted samples, since the degree of substitution has a remarkable effect on the state of solution and the viscosity.

For the determination of the viscosity most investigators use standarized capillary viscometers. For optimum results, the effect of shear rate ($\dot{\gamma}$) should not be overlooked.

In essence, the determination of intrinsic viscosity [η] includes the extrapolation of η_{sp}/c versus $c = 0$ and $\dot{\gamma} = 0$. In performing the measurements the outflow time between two marks is measured for the solution (*s*) and the solvent (*l*) as t_s and t_1, respectively. In the evaluation the following values are calculated:

$$\eta_{rel} = t_s/t_1 \qquad \text{relative viscosity} \qquad (2.7)$$

$$\eta_{sp} = \eta_{rel} - 1 \qquad \text{specific viscosity} \qquad (2.8)$$

$$\eta_{red} = \eta_{sp}/c \qquad \text{reduced viscosity} \qquad (2.9)$$

The shear corrected reduced viscosity can then easily be obtained by graphical extrapolation to zero shear. Such shear corrected reduced viscosities obtained from solutions of varying concentration can then graphically be extrapolated for zero concentration giving the intrinsic viscosity [η] as

$$\lim_{\substack{c \to 0 \\ \dot{\gamma} \to 0}} (\eta_{sp}/c) = [\eta] \qquad (2.10)$$

The intrinsic viscosity of cellulose may also be determined according to the SCAN standard (SCAN CM-15:88). This method is based upon a single point measurement of viscosity and the use of correlation tables. The cellulose is in this method dissolved in 0.5% CED (cupriethylenediamine) solution.

2.4.4 Physical properties and conformation of polysaccharides

One of the most important factors influencing the physical, biochemical and technological properties of biopolymers is the three-dimensional structure of the molecular chain. Ideally, the conformation of a macromolecule can be divided into three idealized groups; sphere, rod-like and random coil.

The radius of gyration (R_G) describes the molecular size and shape in solution. R_G depends of the molecular weight in different ways depending on whether the molecule shape is characterized as spheres, rod-like, random coil or something in between (transition states). The conformation of macromolecules is influenced by several factors like secondary bindings and steric configuration around the bindings between the monomers.

It is possible to define the radius of gyration of a body independent of shape, as the distance from the central of gravity where all mass can be collected without changing the momentum of inertia to the body.

$$I = mR_G^2 \qquad (2.11)$$

where I is the momentum of inertia to the body, R_G the radius of gyration and m the mass. Dividing a body in numerous masses, m_i, each with a distance r_i gives

$$R_G^2 = \frac{\sum_i m_i \cdot r_i^2}{\sum_i m_i} \qquad (2.12)$$

In populations with molecules of the same defined shape, all molecules will have the same radius of gyration. For random coil populations the macromolecules will have different shapes and therefore the radius of gyration has to be defined as an average $<R_G>$. For molecules with the same molecular weight, the radius of gyration for spheres will be small compared to rod-like molecules and random coils. The relationships between radius of gyration and molecular weight for the different shapes is;

$$R_G \propto M^{1/3} \quad \text{spheres} \qquad (2.13)$$

$$R_G \propto M \quad \text{rod-like} \qquad (2.14)$$

$$R_G \propto M^{0.5-0.6} \quad \text{random coil} \qquad (2.15)$$

It is possible to measure the R_G-M relationship by SEC-MALLS for a population of molecules. SEC separates on the basis of molecular size, whereas MALLS gives the R_G and molecular weight for each class of molecules.

2.4.5 Determination of molecular weight and molecular weight distribution

In the commonly used forms, cellulose samples are always polydisperse. This means that they consist of a mixture of molecules having the same basic composition but differing widely in chain length. Thus, molecular weight or the degree of polymerization values must be considered as average values only.

Molecular weight can be determined by various methods: the number average molecular weight ($\overline{M}_n$) from osmotic pressure measurements, weight average molecular weight ($\overline{M}_w$) from light scattering, z-average molecular weight ($\overline{M}_z$) from sedimentation in the ultracentrifuge and the viscosity average molecular weight ($\overline{M}_v$) from viscosity measurements calibrated against one of the absolute methods. The definitions of the various averages of the molecular weight is as follows

$$\overline{M}_n = \frac{\sum n_i \cdot M_i}{\sum n_i} \quad \text{osmosis} \qquad (2.16)$$

$$\overline{M}_w = \frac{\sum n_i \cdot M_i^2}{\sum n_i \cdot M_i} \quad \text{light scattering} \qquad (2.17)$$

$$\overline{M}_z = \frac{\sum n_i \cdot M_i^3}{\sum n_i \cdot M_i^2} \quad \text{sedimentation} \qquad (2.18)$$

$$\overline{M}_v = \left(\frac{\sum M_i^a \cdot c_i}{\sum c_i} \right)^{\frac{1}{a}} \quad \text{viscosity} \qquad (2.19)$$

where n_i is the number of moles of a given fraction i having the molecular weight M_i and a is the MHS exponent.

For a monodisperse sample $\overline{M}_n = \overline{M}_w = \overline{M}_z$ is equal, whereas for a polydisperse sample the different average molecular weight increases in the as follows, $\overline{M}_n < \overline{M}_w < \overline{M}_z$. For $0.5 < a < 1$, $\overline{M}_n < \overline{M}_v < \overline{M}_w$ whereas for $a = 1$, $\overline{M}_v = \overline{M}_w$. It is common to define a polydispersity index, *Pi*, as the ratio between $\overline{M}_w$ and $\overline{M}_n$. For monodisperse samples, *Pi* = 1 and increases with increasing degree of polydispersity. However, *Pi* = 2, may be an indication of a random degradation.

2.4.6 Nuclear magnetic resonance (NMR) spectroscopy

Spectroscopy may be defined as interaction between matter and electromagnetic radiation in such way that energy is absorbed or emitted according to the Bohr frequency condition:

$$\Delta E = h\nu \qquad (2.20)$$

where ΔE is the energy difference between the initial and final states of matter, h is Planck's constant and ν is the frequency of electromagnetic radiation. The type of spectroscopy is classified according to the nature of energy transitions observed and is correlated with the range of frequencies under observation. The energy transitions to nuclei placed in a magnetic field occur in the radio wave region of the electromagnetic spectrum (tens and hundreds of megahertz). The spectroscopy technique that detects this energy transitions is called nuclear magnetic resonance (NMR) spectroscopy.

All nuclei have a property called spin and is characterized by a spin number, *I*. Nuclei whose $I > 0$ have magnetic fields and are NMR active. The actual value of the spin of any

given nucleus depends on the mass number and the atomic number (Table 2.2). Some common nuclei, such as ^{12}C and ^{16}O have $I = 0$ and is therefore NMR inactive.

Table 2.2: Determination of nuclear spin (I) [36]

Mass number	Atomic number	Nuclear spin (I)
odd	even/odd	1/2, 3/2, 5/2, ...
even	even	0
even	odd	1,2,3, ...

Nuclei with nuclear spin have an associated magnetic field and therefore a nuclear magnetic dipole moment, μ. When placed in a external magnetic field, the nuclei will align themselves parallel or anti-parallel to the applied field. The nuclear magnetic moment is directly proportional to the spin by

$$\mu = \frac{\gamma I h}{2\pi} \qquad (2.21)$$

where the magnetogyric ratio, γ, is a constant for each nucleus. A nucleus of spin I has $2I+1$ possible orientations, which are given by the magnetic quantum number, m_I. The energy of interaction is proportional to the nuclear moment and the external applied field, B

$$E = \frac{\gamma h}{2\pi} \cdot m_I B \qquad (2.22)$$

Since the external magnetic field and the magnetic dipole is not parallel, the external field will try to align the magnetic dipole. The torque between the external magnetic field and the nuclei creates a rotation around the axis of the external magnetic field. This determines the resonance frequency given by the Larmor equation

$$\nu_0 = \frac{\gamma B_0}{2\pi} = \frac{(E_\alpha - E_\beta)}{h} \qquad (2.23)$$

The lower energy for the nucleus aligned with the external field, the α-state, is more populated than the β-state. The numbers of nuclei in each spin state are described by the Boltzman distribution

$$\frac{N_\beta}{N_\alpha} = e^{-\gamma B_0 \frac{h}{2\pi k T}} \qquad (2.24)$$

where the N values are the number of nuclei in the respective spin states, h is Planck's constant, k is the Boltzman constant, and T is the temperature. Summing all vectors from the nuclear spins will therefore create a resultant vector pointing in the direction of the applied field.

If a coherent magnetic field with the resonance frequency is applied to this system the different spins will themselves be coherent and the population levels will start changing. Magnetization in the x,y-plane will be created precessing with the Larmor frequency. It is this magnetization that give rise to what we call a resonance line in an NMR spectrum.

In a homogeneous system with only one kind of nucleus, the NMR spectrum will show only a single peak at a characteristic frequency. In real samples the nucleus is influenced by its environment. Some environments will increase the energy separation of the spin-states giving a spin transition at a higher frequency. Others will lower the separation consequently lowering the frequency at which the spin transition occurs. These changes in frequency are called the chemical shift of the nucleus. By examing the exact frequencies (chemical shift) at which the spin transitions occur conclusions about the nature of the various environments can be made. This environment is influenced by e.g. chemical bonds, molecular conformations and dynamic processes.

Application:

Although NMR has been utilized for composition analysis as well as structural characterization of polymers, the application is limited according to the relatively low solubility and high viscosity of solutions of these materials. Molecules and molecular groups in solids or semisolids are generally not subject to rapid isotropic molecular tumbling as in non-viscous solutions. Spectra of these samples typically reveal broad lineshapes with lack of fine structure. Molecular degradation may be used in the determination of degree of substitution on polymers (Chapter 3.4.1).

Another way to deal with this is by using high resolution magic angle spinning (HR-MAS). In this technique the dipolar interactions and chemical anisotrophy are averaged randomly to zero by a specific angle ("magic angle") for which $3\cos^2\theta - 1$ is zero ($\theta = 54.7°$) This is done by mounting the sample in the rotor at the magic angle with respect to the magnetic field direction. The linewidth can also be decreased by rotating a semisolid sample around its own axis at high speed (several kHz).

For nucleus with spin-1/2 and low magnetogyric ratios (^{13}C) in solids (cellulose), the linebroadening due to dipolar interactions with e.g. protons is largely overcome by high-power double irradiation. A combination of MAS and high-power decoupling reduces the linewidth further. However, this way of obtaining solid-state spectra has certain drawbacks. The rather long spin lattice relaxation time (T_1) for ^{13}C means that the pulse rates have to be sufficiently low and a long accumulation times are needed. This disadvantages can be curciumvented by permit an exchange of polarization between the ^1H and ^{13}C spins. This magnetization transfer is called "cross-polarization" and in combination with MAS, the whole is abbreviated to CP-MAS. This methods permit solid substances to be studied at reasonable high resolution. A comparison of chemical structure in solution and in solids may then be obtained. The crystallinity index of cellulose may be determined by CP-MAS.

Comparison of crystallinity measurement by NMR and X-ray

CP-MAS measures the numbers of carbon atoms in a particular environment. Because only material within the crystallites will appear as crystalline in the NMR spectrum, the crystallinity index will depend on the size of the crystallites. In addition, the NMR method is sensitive to short-range ordered crystallites.

In contrast to the NMR technique, X-ray diffraction measures the fraction of molecules which are arranged in a regular repeating pattern. To be "seen" as crystalline by X-ray, the crystallites must be of a certain minimum size. Materials on the surface of the crystallites, which will be of greater mobility and a lower degree of order than the material in the interior of the crystallites, will be measured as crystalline material. In addition, small defects within crystalline regions will be included within the crystalline fraction if they are too small to be seen as regions of disorder. Crystallite size differences and lattice distortions result in underestimates of crystallinity if the crystalline standard is not similar to the crystalline components of the samples.

CHAPTER

3

EXPERIMENTAL PROCEDURES

3.1 Analytical materials

A list of the chemicals used in this work is found in Appendix A.1. Characteristics of the cellulose samples (acid sulfite pulps, cotton linters and carboxymethyl celluloses) are given in Appendix A.4. A set of polystyrene standards was used in measuring the column stability and reproducibility as well in the comparison between different column materials. A list of this standards is found in Appendix A.2.

3.1.1 Cellulose materials

Samples included eight spruce sulfite pulps (D1-D8) from Borregaard Industries, Ltd. and four commercial grades of cotton linters (C1-C4) obtained from Peter Temming AG. In addition, one sample of microcrystalline cellulose (MCC) was produced at the laboratory of Borregaard Industries, Ltd.

3.1.2 Production of carboxymethyl cellulose

Three different methods for carboxymethylation have been used in the synthesis of carboxymethyl cellulose (CMC) samples, namely heterogeneous, heterogeneous-II and homogeneous carboxymethylation. The conditions for the three methods are given in Table 4.6 and 4.8.

Heterogeneous carboxymethylation

The celluloses were carboxymethylated in nitrogen atmosphere by the use of the standard solvent method with monochloroacetic acid (MCA)/NaOH in an isopropanol/ water mixture [37]. 5 g of dried cellulose in 150 ml of isopropanol was stirred vigorously, while 15 ml of aqueous NaOH (1-2 mol NaOH/AGU) was added dropwise within 30 min. at room temperature. Stirring was continued for another hour, and monochloroacetic acid (1.5-2.0 mol MCA/AGU) dissolved in water was then added over a period of 30 min. The mixture was stirred for 2.5-4.0 h on a silicon oil bath at 75 °C. The mixture was cooled, filtrated, suspended in isopropanol and neutralized with acetic

acid. The CMC product (Table 4.8) was collected and filtered, washed with isopropanol and several times with methanol before soxhlet extraction with pure methanol for 2 hours. The CMC samples were freeze dried before storage under argon atmosphere. A double carboxymethylation was performed by repeating the procedure.

Heterogeneous-II using 1,1,3,3-tetramethylurea and dimethyl sulfoxide

In order to obtain CMC samples of higher DS in a shorter reaction time 1,1,3,3-tetramethylurea (Me_4U) and dimethyl sulfoxide (Me_2SO) were used as a promoter of carboxymethylation of cellulose [38]. One part (by wt.) of cellulose powder and 30 parts (by vol.) of Me_2SO-Me_4U (1:1, mol ratio) were stirred for 30 min. at 30 °C. 1/3 of the total volume of 50% NaOH (aq.) was added to the cellulose suspension under vigorous stirring during 10 min. 1/3 of the total amount of 50% aq. monochloroacetic acid was then added. After stirring for 30 min., the process was repeated twice. The molar ratio of the reagents per mol of D-glucosyl residue of cellulose was varied according to Table 3.1. The mixture was transferred to a 200 ml round bottomed flask fitted with a reflux condenser and heated under continuous stirring for 3 h at 75 °C to complete the reaction. The mixture was dialyzed against deionized water for 48 h. The solution was freezed dried before storage under argon atmosphere.

Table 3.1: Molar ratios of reagents and DS value of CMC samples.

Solvent	NaOH	MCA	DS of CMC samples
Me_2SO/Me_4U (1:1)	2	2	1.2
	4	4	1.9
	8	8	2.4

Homogeneous carboxymethylation

Initially, the dissolution of cellulose (5g) was performed according to the method described in Chapter 3.2.1. The homogeneous carboxymethylation was performed according to the procedure described by Heinze et al. [39]. To the dissolved cellulose sample, a suspension of MCA (5 MCA/AGU, 45 °C) in 20 ml DMAc was added within 20 min. followed by a suspension of 9.8 g dried, pulverized NaOH (45 °C) in DMAc within 20 min. under vigorous stirring at room temperature. The temperature was raised to 70 °C. After 4 h the reaction mixture was cooled to room temperature and precipitated into 300 ml ethanol. The precipitate was collected by filtration, dissolved in 100 ml distilled water, neutralized with acetic acid and re-precipitated into 300 ml ethanol. The product was filtered and washed with ethanol several times before freeze drying and storage under argon atmosphere. A double carboxymethylation was performed by repeating the procedure.

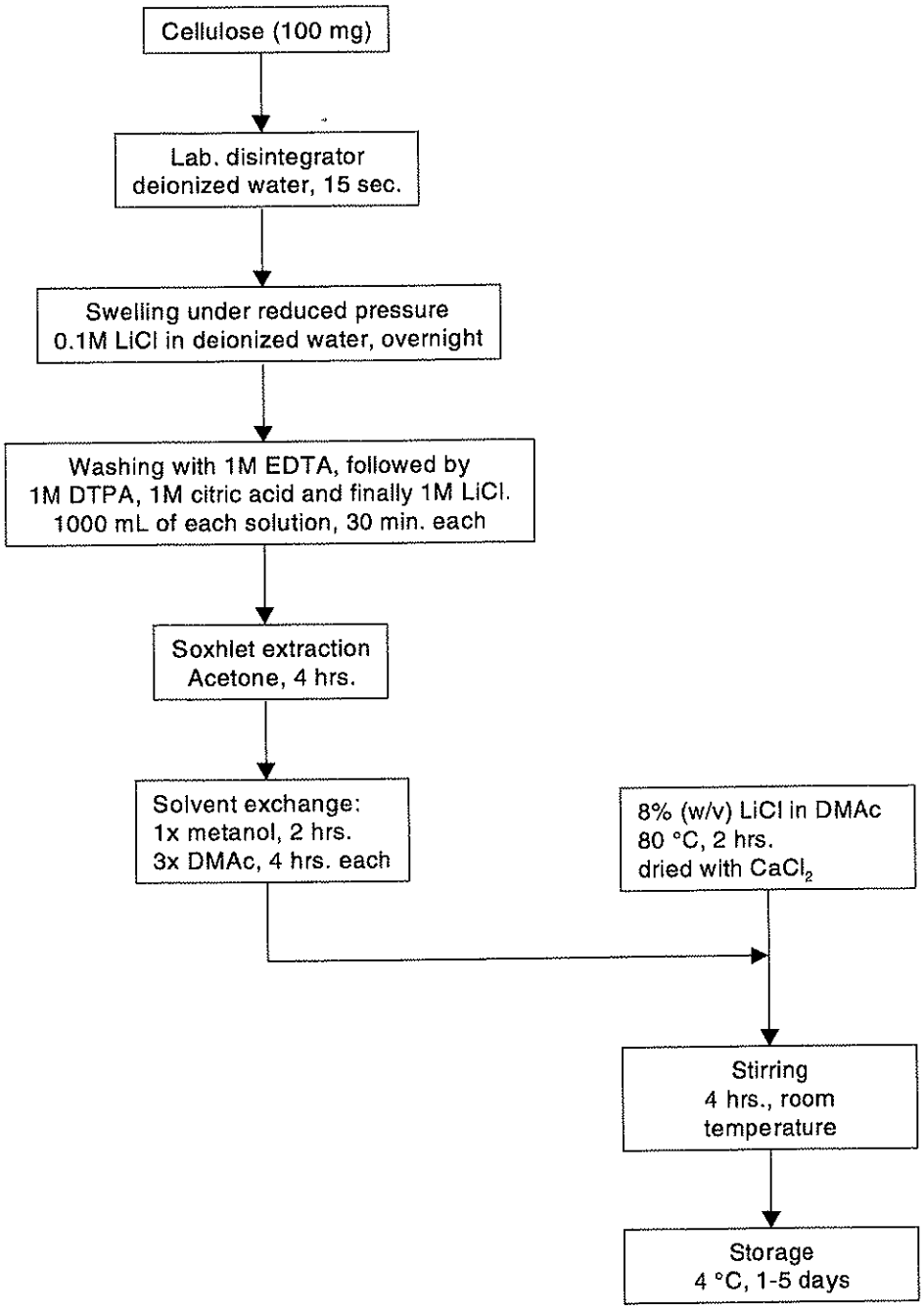

Figure 3.1 Schematic description of the different steps of dissolving cellulose in 8% LiCl/DMAc.

3.2 Sample preparation

3.2.1 Dissolution of pulp samples in LiCl/DMAc

The pulp samples were disintegrated in deionized water using a laboratory disintegrator. The samples were then swollen in 0.1M LiCl under vacuum overnight and subsequently washed with 2% aqueous EDTA, 2% aqueous DTPA and finally with 5% aqueous citric acid. The cellulose was further washed with 0.1M LiCl followed by an acetone extraction. The sample was then solvent exchanged with methanol and subsequently with DMAc under absolute dry conditions by Soxhlet extraction. Simultaneously, an 8% solution (w/v) of LiCl in DMAc was made by continuous stirring and heating to approximately 80 °C. The pulp was directly added to the 8% LiCl/DMAc solution and continuously stirred for 4 hours at room temperature. The solution was then allowed to stand for up to 5 days at 4°C to achieve complete dissolution of the cellulose. The sample concentration is 16 mg/ml in 8% LiCl/DMAc. Figure 3.1 shows a flow diagram of the final dissolution procedure of acid sulfite pulps and cotton linter cellulose (Appendix A.3).

3.2.2 Dissolution of CMC for SEC analysis

The samples were dissolved directly in deionized water and stirred for at least 2 hours at 40 °C prior to use. The samples were dialyzed against deionized water for 72 hours. Insoluble particles were removed by high pressure filtration of the solutions through a 0.7μm Whatman GF/F filter. To measure the sample concentration after dialysis, a DOC (dissolved organic carbon) analysis was performed on a DOC-analyzer (Dohrman, DC-190).

Before injection into the SEC column, the samples were diluted to a 0.05M Na_2SO_4 solution with 0.01M EDTA and filtrated through a 0.8 μm Millex/AA filter. The pH value of the samples was measured to pH~6.2.

3.2.3 Hexose content (Phenol-sulfuric acid analysis)

A phenol-sulfuric acid analysis was included to measure the lowering in concentration by the initial filtration before the SEC analysis. The analysis is based on the method described by Dubois [40]. 2 ml of the CMC sample solution was pipetted to a calorimetric tube. 0.5 ml 3% phenol and 5 ml of concentrated sulfuric acid was added and mixed properly. After 30 min. the tube was cooled in cold water. The absorbance was measured at 485 nm against a reference (blank) sample, which was made by the same procedure, but changing 2 ml of the CMC sample with 2 ml water. 4 parallels were performed using the same stock solution of CMC (initial concentration: 1mg/ml and diluted by 1:50)

3.3 SEC experiments

3.3.1 Determination of refractive index increment for cellulose

Refractive index increments, $(dn/dc)_\mu$, of spruce sulfite and cotton linter cellulose in 0.5% LiCl/DMAc were determined at 40°C using a Shimadzu differential refractometer

(Shimadzu RID-10A) which was connected to a thermostatic water bath. The instrument was calibrated with KCl and polystyrene solutions with known refractive index.

Low molecular weight samples of cellulose (D1, 80 μg) with exact known concentration were injected onto the columns without prior filtration. The $(dn/dc)_\mu$-values were calculated automatically using the ASTRA software (Wyatt Technologies.) by assuming 100% recovery of the injected material and known calibration constant for the RI-detector (RI_c):

$$\frac{dn}{dc} = \frac{\sum V_i \cdot RI_c}{\sum c_i} \qquad (3.1)$$

where V is the RI detector output voltages and c is the polymer concentration. The measured value of $(dn/dc)_\mu$ was then used in the SEC/MALLS/RI experiments.

3.3.2 SEC separation of cellulose

Before injection, the solution was diluted to 0.5% DMAc/LiCl and filtered through a solvent resistant, disposable teflon filter (Millex SR 0.5mm and Millex CN 3.0 mm for the highest molecular weight cellulose, both filters from Millipore).

SEC was performed at 40°C using a Perkin Elmer (Series 2000) HPLC pump operating by a flow rate of 1.0 ml/min and with a autosampler. Injected samples contained 50-200 μg of dissolved cellulose. The elution was monitored by a refractive index detector (Shimadzu RID-10A) and a multi angle laser light scattering detector (MALLS, Wyatt Dawn DSP, 633 nm) equipped with an in-line filter holder (Millipore) with 0.2μm PTFE-filter (Fluoropore-FG, Millipore). The refractive index increment, $(dn/dc)_\mu$, was taken to be 0.104 (**Paper I**). Data acquisition and molecular weight calculations were performed by the ASTRA software (Wyatt Technologies).

3.3.3 SEC separation of carboxymethyl cellulose

The samples were analyzed by SEC (columns TSK G6000PWXL, G5000PWXL and G4000PWXL, serially connected; 0.75 · 30 cm, flow rate of 0.5 ml/min.) combined with low angle laser light scattering (LALLS) instrument (KMX-6, Chromatix) [41]. The SEC-LALLS was performed at room temperature. Three parallels of the same stock solution (injection volume 250 μl containing approximately 1.0 μg/μl) were performed. The elution was monitored by a refractive index detector (Shodex RI SE-61). and a low angle light scattering photometer (chromatic KMX-6). Data acquisition and molecular weight calculations were performed using the PCLALLS software. The number (M_n) and weight average ($\overline{M}_w$) molecular weights were determined by extrapolating the linear regions in the calibration plots (log M against V) to the total column volume. The value of the refractive index, *dn/dc*, was not measured but set to 0.150 for internal comparison.

3.4 NMR measurements

3.4.1 Determination of DS by NMR

The CMC samples were hydrolyzed using trifluoroacetic acid (TFA). About 150 mg of a CMC sample was weighed into a 5 ml glass vial. 1 ml of D_2O was added and allowed the CMC to swell. Subsequently, 1-2 ml of a 50/50 (v/v) mixture of D_2O + TFA was added slowly. The slurry was heated to 85 °C and stirred for 5 hours to disperse the CMC sample as completely as possible. The resulting solution was neutralized with dilute sodium deuteroxide (NaOD). The water was removed by freeze-drying (with D_2O twice) and the CMC residue was dissolved in D_2O to a concentration of 0.5% w/w for ^{1}H-NMR spectroscopy. All samples were centrifuged and filtered to remove aggregates and undissolved CMC material before recording the NMR spectrum. The pH was adjusted to 1 by adding TFA. 3-(trimethylsilyl)-propionicacid-d_4 sodium salt (TSP) was used as an internal reference.

^{1}H-NMR spectra (Figure 3.2) were measured in 5mm OD-tubes (Wilmad) at a probe temperature of 7 °C on a Bruker Avance DRX600 spectrometer with an operating proton frequency of 600 MHz. The spectra were acquired with a modified 1D NOESY-presaturation pulse program with double presaturation on water. The flip angle was 60° with a repetition delay of 2s. The degree of substitution was determined according to Ho et al. [42].

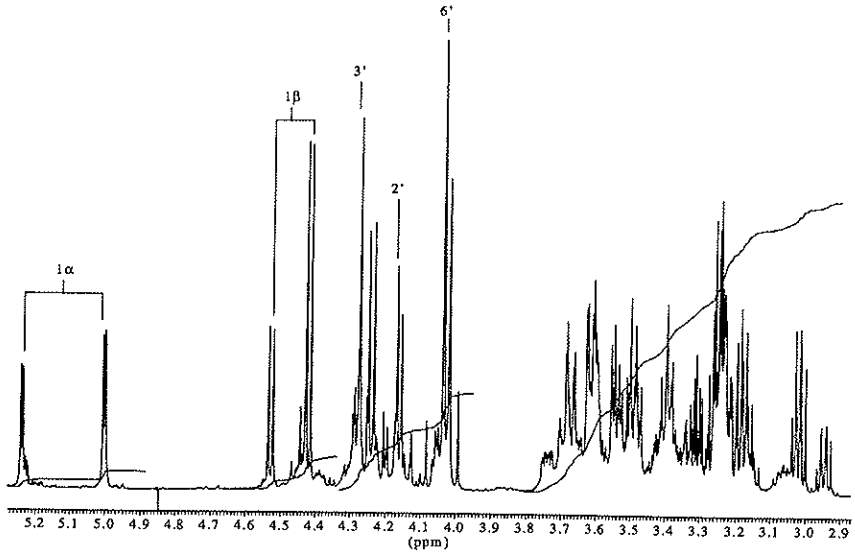

Figure 3.2 ^{1}H-NMR spectrum of a selected CMC sample.

3.4.2 Determination of cellulose crystallinity by NMR

The ^{13}C-CP/MAS-NMR spectra were recorded on a BRUKER AMX200 instrument operating at 50.3 MHz (^{13}C) using a double air bearing probe and 5 mm ZrO_2 rotors. The spinning rate was 5.0 kHz, contact time 1.0 ms, acquisition time 0.35s, sweep width 220 ppm and a relaxation delay of 2.5s. For each spectrum 2048 transients were accumulated with 2k data points and zero filled to 4k data points. The spectra were referenced to the carbonyl in external glycine (δ 176.03 ppm).

3.5 X-ray measurements

3.5.1 Determination of crystallinity by X-ray

A Siemens instrument (LOFTE) using nickel-filtered copper K_α radiation generated at 50 kV and 160 mA was used. The source and slits were 0.6 mm and 0.4 mm, respectively. Angular scanning was continuous from 5° to 60° at 1° /min. The experimental data was processed by the Origin programme for technical graphics and data analysis. For the determination of crystallinity, X_{crist}, the Ruland's method was used [43].

CHAPTER

4

RESULTS AND DISCUSSION

4.1 Overview

The first part of this chapter contains research on dissolving high alpha-cellulose spruce sulfite pulps and cotton linters in LiCl/DMAc, column materials for SEC separation, MWD of the investigated cellulosic materials and consideration of the dissolved cellulose molecular shape.

The second part is a characterization study of CMC with emphasis on how different raw materials and synthetic pathways influences the MW, the MWD and the chemical structure of CMC. The analytical methods used, are SEC-LALLS, NMR spectroscopy and X-ray diffraction.

4.2 Molecular weight distributions of cellulose samples (Paper I-II)

The purpose of studying the molecular weight distribution of cellulose material is the need for characterizing the raw material before cellulose modification. In this way it is possible to study the structural and chemical changes during derivatization reactions.

Due to the low solubility, MWD analysis of cellulose by SEC has not been a trivial task. Problems like incomplete dissolution, aggregate formation [44] and precipitation of the cellulose on the column matrix causing column plugging/adsorption has been reported [45-47]. These problems may be eliminated by choosing more suitable column materials (**Paper I**) and by better solubility. The latter requires improvements in the procedure and conditions for dissolution of cellulose in LiCl/DMAc.

4.2.1 LiCl/DMAc

As mentioned in Chapter 2.1.3, cellulose is insoluble in most solvents because of the intra- and intermolecular hydrogen bonds. Several chemicals have the ability to swell and to more or less dissolve cellulose, but this involves in most cases a considerable

chain degradation. Lithium chloride/*N,N*-dimethylacetamide (LiCl/DMAc) is considered to be a non-degrading solvent for cellulose [48,49] although a slight decrease in viscosity has been reported after 30 days [30].

4.2.2 Dissolving cellulose in LiCl/DMAc

The optimization of the dissolving conditions of wood pulp cellulose in LiCl/DMAc is highly dependent of the molecular weight, the crystallinity and the lignin content [50]. In the case of cotton and spruce cellulose, minor alterations in the procedure can mean the difference between success and failure. Variations in the existing procedures for dissolution were tested in this work but a direct application was unsuccessful for a total dissolution, especially of high molecular weight samples. A new modified method was required for the characterization of the acid sulfite pulps.

Description of the modified dissolution procedure:

Metal ions are naturally associated to native cotton and wood by ionic bonding to carbohydrates. The formation of the Li^+-(DMAc)-Cellulose-Cl^- complex may be hindered by these ions. Remaining ions after pulping were removed by a combination of complex binders (EDTA, DTPA and citric acid). By the same reason deionized water was used in the disintegration, swelling and washing steps. The introduction of a separate LiCl washing step was introduced to make sure that ions associated with the pulp were Li^+ ions. An acetone extraction was performed to remove possible extractives left in the pulp.

The solubility of cellulose increases as the LiCl concentration increases [30]. A sample concentration from 0.5-1.5 mg/ml (cellulose/DMAc) was tested and compared with the concentration of LiCl (6, 8, 9 and 12%) in the solvent system. With LiCl concentration of 8%, a complete dissolution of cellulose occurred. As found in the literature a higher LiCl concentration is only needed for highly crystalline samples [23].

According to the literature, longer activation and stirring times are necessary for cellulose with high crystallinity (α-cellulose content), MW and lignin content [50]. The suspension of cellulose-LiCl/DMAc was stirred for 4 hours before the sample was allowed to stand for 1-5 days at 4 °C [51] to obtain complete dissolution. Figure 3.1 shows a flow diagram of the further developed dissolution procedure of acid sulfite pulps and cotton linter cellulose used in the SEC analysis.

4.2.3 Column matrix

In the search for a more suitable column matrix, two types of macroporous monodisperse particles (MMP), designed I and II were studied in comparison to the commercial PLgel Mixed B from Polymer Laboratories. Particle I has a higher proportion of small pores and should therefore have the potential to separate a lower molecular weight than particle II.

Polystyrene standards were used for assessing and comparing the separation ranges of the different particles. The calculated calibration curves (log M versus elution volumes at

the peak maximum) of columns I, II and the combination of II+I are shown in Figure 4.1, which also includes a comparison with the PLgel Mixed B column. The serial combination of column II+I gave good separation across the entire molecular weight range and was therefore used in the separation of cellulose. Due to the lack of available standards with molecular weights larger than $2.0 \cdot 10^6$, the full separation potential could not be directly assessed in the high molecular weight range for the present macroporous monodisperse particles.

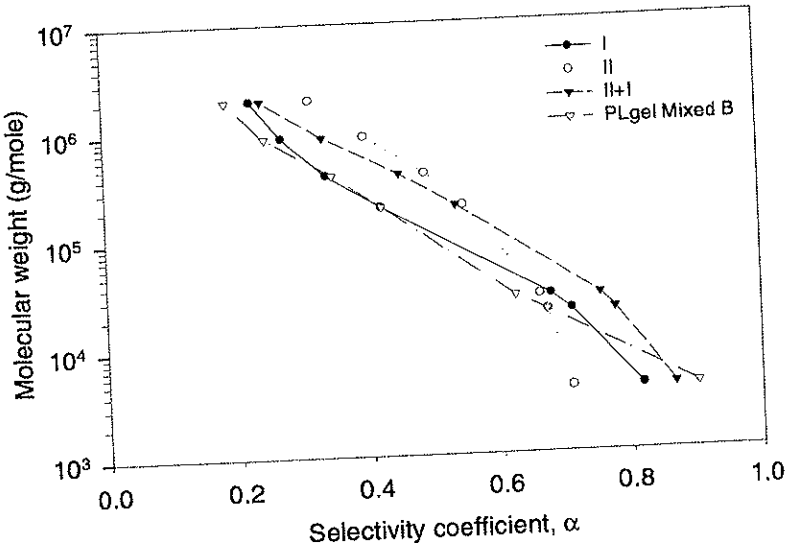

Figure 4.1 Calibration plot of polystyrene standards for column I, II, II+I and PLgel Mixed B

Favourable chromatographic properties generally associated with monosized particles were obtained, and column packing as well as the separations were performed with very low backpressures (150 psi at 1.0 ml/min.) compared to PLgel Mixed B (1060 psi at 1.0 ml/min.). Generally, more pronounced increases in the back pressure, and even column plugging, was observed for the with PLgel Mixed B column. Only 1% variation in calculated molecular weight and retention time for column II+I was observed after several hundred injections of cellulose interspersed with washing procedures.

4.2.4 Measuring $(dn/dc)_\mu$ for cellulose in 0.5% LiCl/DMAc

By the dissolution of materials the refractive index of the solution is changed by an amount proportional to the concentration. This proportionality factor is called the refractive index increment, $(dn/dc)_\mu$, and is roughly independent of molecular weight.

The $(dn/dc)_\mu$ value is one of the parameters required to compute molecular weight from light scattering data. The value based on the average of 6 injections was calculated to 0.104 ± 0.002 ml/g for cellulose in 0.5% LiCl/DMAc at 40°C.

4.2.5 SEC separation of cellulose by MMP

Figure 4.2 shows RI signals and calibration plots (plots of log *M* versus elution volume as calculated using the ASTRA® software) obtained for two separate preparations (one filtered and one unfiltered) of the same cellulose stock solution (D1, 8% LiCl in DMAc) with 2 parallels each. The sample recovery was calculated to 100%, indicating that no significant adsorption of the cellulose material took place. Figure 4.2 shows good reproducibility and the molecular weight was estimated to $(2.12 \pm 0.10) \cdot 10^5$ g/mol. No significant differences between the filtered and unfiltered sample were observed.

The elution profiles and the corresponding calibration curves were independent of the amount of sample injected (0.2-1.0 mg/ml), although the signal-to-noise ratio of the light scattering signal decreases with decreasing sample concentration (Figure 4 in **Paper 1**). The calculated $\overline{M}_w$ was also independent of the injected amount of the sample.

The MMP was further tested for the molecular weight separation of three high purity cellulose samples of different origin, microcrystalline cellulose, acid sulfite pulp cellulose and cotton linters cellulose (MCC, D1 and C4 respectively). Figure 4.3 shows that the samples were well separated, as demonstrated by the linear decrease in log *M* with increasing elution volume. The $\overline{M}_w$ values of the three samples investigated (MCC, D1 and C4) were calculated $5.3 \cdot 10^4$, $2.0 \cdot 10^5$ and $8.0 \cdot 10^5$, respectively. The low-

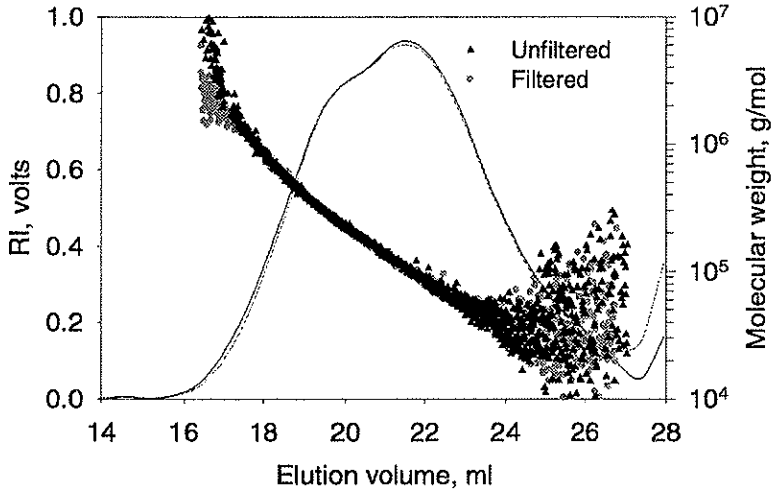

Figure 4.2 Chromatograms and calibration plots of filtered and unfiltered injection of the same cellulose sample.

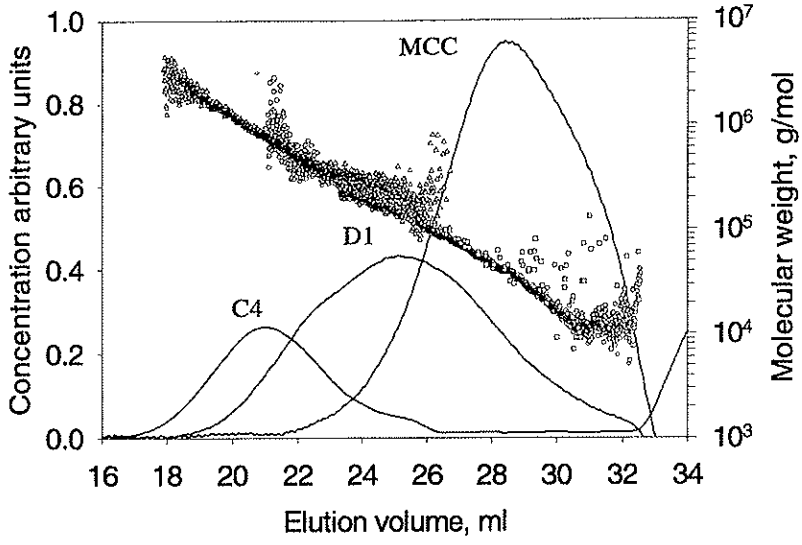

Figure 4.3 Calibration plots and elution curves for the cellulose samples C4, D1 and MCC

molecular part of MCC seemed not to be fully separated from the salt peak (Figure 4.3). However, the calibration plot is decreasing linearly with increasing elution volume for the entire sample. The column generally demonstrates good separation according to M in the range from $2.0 \cdot 10^4$ to $2.0 \cdot 10^6$ for cellulose and is supported by overlapping calibration plots.

During this work the MMP columns have been installed and used at Borregaard Laboratories. Lately, they have been involved in an interlaboratory comparison of different SEC methods [52] for the determination of molecular weight distribution of cellulose. The preliminary results shows that there exists differences in the data obtained by the research groups [53]. For all the cellulose samples lab 2 (Borregaard Lab.) gives higher values of average molecular weight (Table 4.1). The differences are considerably higher for the high molecular weight cellulose (VHV-S from Borregaard Ind. Ltd.). This indicates that the MMP columns separates cellulose of higher molecular weights. The MALLS data shows no sign of aggregates which reduces the possibility that the high average molecular weight is influenced by the molecular weight of aggregates. This means that the other columns probably underestimate the content of high molecular weight cellulose molecules. As mentioned in Chapter 4.2, adsorption to the column material is a known problem in SEC analysis of cellulose. In addition, long chain cellulose molecules are more difficult to dissolve. It is thus most likely to believe that the

adsorption of cellulose to the column material is the reason why the measured molecular weight is differing between the laboratories.

Table 4.1: Interlaboratory (1-5) comparison of $\overline{M}_w$ ($\cdot 10^3$ g/mol) determined by different SEC methods (redrawn from Schelosky et al. [53])

Lab	1[a]	2[b]	3[c]	4[d]	5[e]
BKZO3	226	410	368	228	387
Solucell	149	270	265	202	274
Avicell	30	54	48	39	-
Viscose Fibre	55	67	90	58	91
Bor. SVS	251	480	384	247	408
Bor. VHV-S	686	1550	707	568	824

a. LiCl/DMAc, MALLS
b. LiCl/DMAc, MALLS (Borregaard)
c. Tricarbanilate, THF, MALLS
d. Tricarbanilate, THF, RALLS-Visco
e. Nitrate, THF, MALLS

4.2.6 Molecular weight distribution of acid sulfite pulps

Softwood tracheids ("fibers") consists of approximately 40% cellulose, while the remainder of the fiber components are hemicelluloses and lignin. A strong chemical treatment is thus necessary to give a pulp with a high content of cellulose. In the acid sulfite process the wood raw material is treated with a strongly acidic cooking liquor consisting of aqueous SO_2 and Ca-bisulfite at high temperatures (120-150 °C). Under acidic conditions, one will have a non-random hydrolytic attack on the amorphous regions of the cellulose molecules, and a broad molecular weight distribution will be expected [54]. This is clearly demonstrated in Figure 4.5. In addition, the fiber wall of the tracheids has a comparatively larger proportion of amorphous hemicelluloses and lignin in the outer parts of the cell wall. Thus, acidic hydrolysis will preferably take place in the outer parts of the fiber wall and a further broadening of the molecular weight distribution should be expected (Figure 4.4). This broadening in MWD can be seen in Figure 4.5. The bimodal distribution of the high molecular weight pulps like samples D7 and D8 would be expected for a non-random degradation where a certain fraction of the cellulose molecules is subjected to strong degradation, while another fraction is in some way protected from acidic hydrolysis.

In order to obtain estimates of the MWD, it is necessary that the calibration curve is correctly assigned across the entire distribution. This is usually not the case at the low molecular weight tail because of increasing signal-to-noise in the light scattering signal and this results in an overestimation of the molecular weight in this area. This is probably

the explanation of the low molecular peak in Figure 4.5 at molecular weight of $1.0 \cdot 10^4$ to $2.0 \cdot 10^4$. However, the low-molecular weight peak may also be attributed to hemicelluloses, which are reported to have significantly lower molecular weights than reported for cellulose [4].

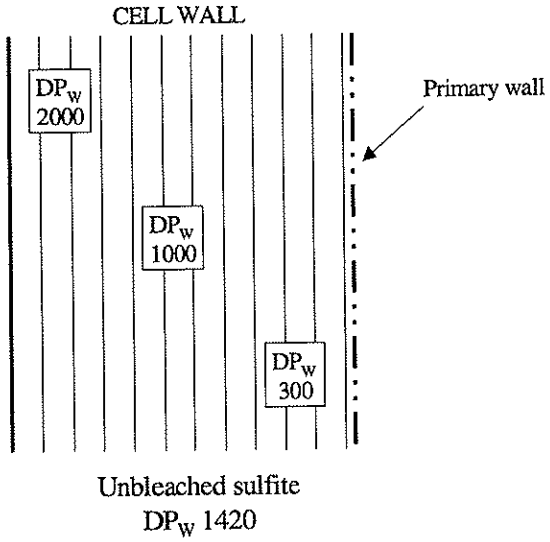

Figure 4.4 Principal DP distribution across the fiber wall of sulfite pulp carbohydrates [9].

Figure 4.5 shows the molecular weight distributions of seven acid sulfite pulp samples. One sample (D5) has been omitted from this figure due to different process conditions during manufacture. The other samples have been manufactured using the same chemical composition of the cooking liquor and the same sequence of bleaching chemicals, although with slightly different process conditions in order to produce pulps with different average molecular weight. These samples should thus be directly comparable. One can notice that together with the shift in average molecular weight to lower values, a slight trend towards a narrower MWD can be observed.

The calculated $\overline{M}_w$ value and polydispersity index (Pi, $\overline{M}_w/\overline{M}_n$) of every acid sulfite pulp sample analyzed is shown in Table 4.2. The sample recoveries were calculated to 100% for low molecular weight cellulose and $92 \pm 2\%$ for high molecular weight cellulose. MALLS data shows little light spreading which indicates that the cellulose has been well diluted.

The large difference in molecular weight distribution between the cotton linters and spruce sulfite pulp samples illustrated in Table 4.3 can be explained by considering differences in both fiber morphology and process conditions during manufacture for the two types of cellulose. Cotton linters consist predominately of highly crystalline cellulose, and only mild alkaline digestion and bleaching is required to give a cellulose

product of acceptable purity. Cotton linter cellulose has a narrower MWD due to the mild process conditions and high structural homogeneity compared to acid sulfite pulp.

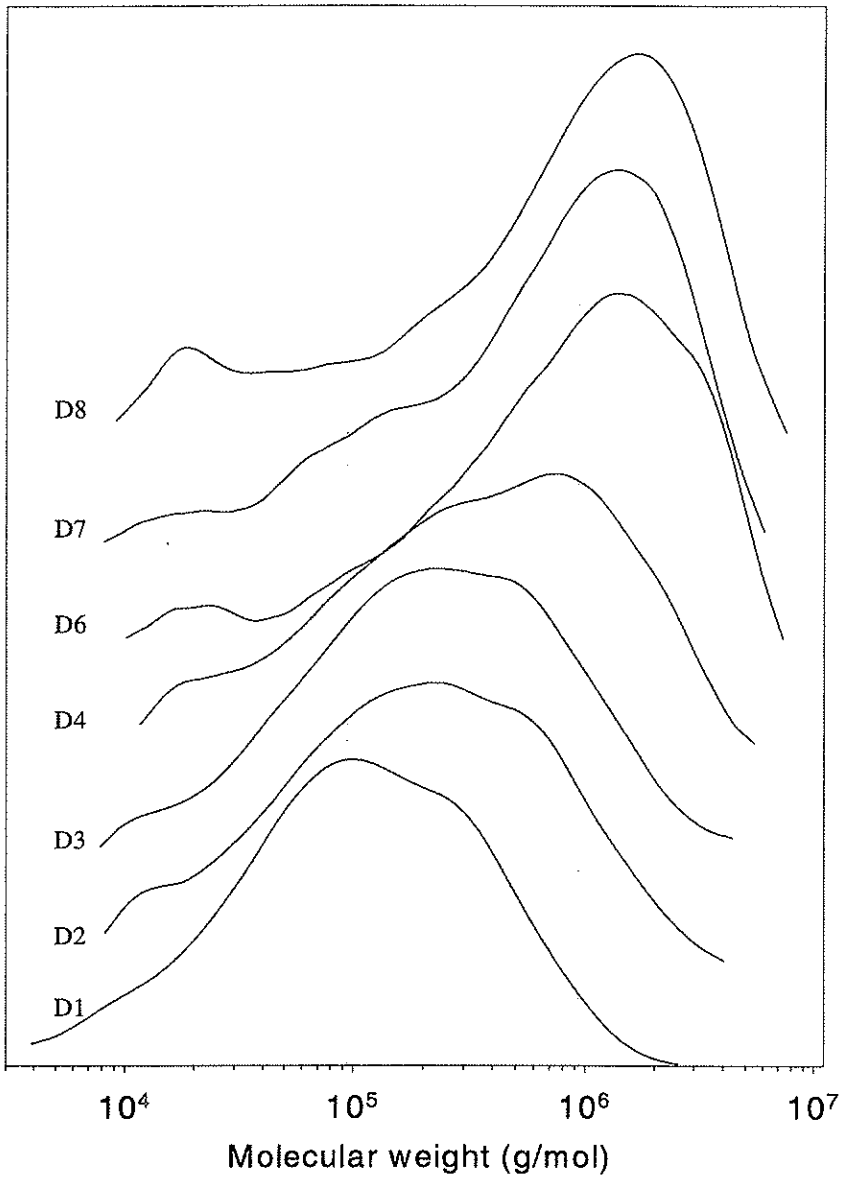

Figure 4.5 Molecular weight distribution of acid sulfite samples.

Table 4.2: Weight-average molecular weight, $\overline{M}_w$ and polydispersity index, $\overline{M}_w/\overline{M}_n$, of spruce sulfite pulp samples, calculated from SEC-MALLS data

Sample	$\overline{M}_w$ ($\cdot 10^3$) g/mole	$\overline{M}_w/\overline{M}_n$
D1	240	3.0
D2	400	5.4
D3	450	2.4
D4	690	5.5
D5	1030	5.4
D6	1300	3.0
D7	1450	5.7
D8	1550	4.9

It is shown that for similar intrinsic viscosity and thus similar $\overline{M}_v$, the acid sulfite pulps have a higher weight average molecular weight and a lower $\overline{M}_n$ than cotton linter celluloses. This would be expected for a polymer with a MHS exponent (a) between 0.5 and 1.0.

Table 4.3: Calculated $\overline{M}_w$, $\overline{M}_n$ and $\overline{M}_w/\overline{M}_n$ for spruce sulfite and cotton linter cellulose with comparable intrinsic viscosities

Intrinsic viscosity	Acid sulfite pulp			Cotton linter		
$[\eta]_{SCAN}$ (ml/g)	$\overline{M}_w$ ($\cdot 10^3$)	$\overline{M}_n$ ($\cdot 10^3$)	$\overline{M}_w/\overline{M}_n$	$\overline{M}_w$ ($\cdot 10^3$)	$\overline{M}_n$ ($\cdot 10^3$)	$\overline{M}_w/\overline{M}_n$
380	240	80	3.0	180	100	1.8
1200	1030	191	5.4	890	330	2.7
1550	1550	316	4.9	1370	685	2.0
1900	-	-	-	1750	1167	1.5

4.2.7 Consideration of cellulose molecular shape

Figure 4.6 shows the relationship between molecular weight and radius of gyration, R_G, for each SEC elution slice. A linear regression of the data provides the scaling factor (α) which was calculated to 0.55 for cellulose in 0.5% LiCl/DMAc. The relation between molecular weight and radius of gyration can thus be expressed by

$$R_G \propto M^{0.55 \pm 0.01} \qquad (4.1)$$

For random coils in good solvents [55], the exponent α in equation 4.1 varies between 0.5 and 0.6, while higher values of α ($0.6 < \alpha < 1.0$) are considered an indication of a stiff coil or rod-like conformation. According to the classical theory of polymers, cellulose can be considered to be a random coil.

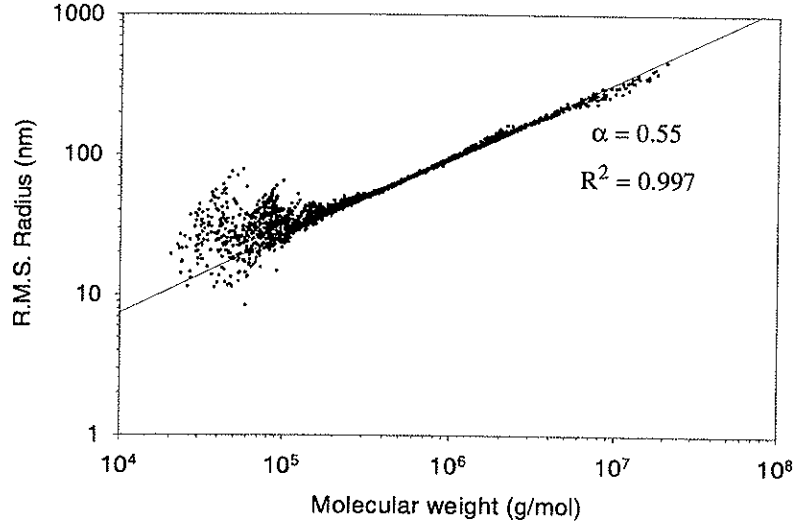

Figure 4.6 Plot of radius of gyration (R_G) versus molecular weight.

The determination of intrinsic viscosity is experimentally more easy than determination of molecular weight and viscosity measurements are often preferred for molecular weight determination of polymer samples. However, when the R_G versus M relation is known, the equivalent sphere model for polymer solution viscosity [56] can give the relationship between the radius of gyration and the intrinsic viscosity of a polymer:

$$[\eta] \propto \frac{R_G^3}{M} \qquad (4.2)$$

The Mark-Houwink-Sakurada (MHS) equation, Eq. 2.6, gives the relation between intrinsic viscosity and molecular weight for a polymer. Here, a random coil will have a values ranging from 0.5 at θ-conditions to 0.8 in good solvents. The exponent in the MHS equation can thus be estimated from SEC-MALLS data. Given that for cellulose in LiCl/DMAc (0.5% LiCl) the radius of gyration depends on the molecular weight as given

in Eq. 4.2, the MHS equation can be expressed by Eq. 4.3, indicating that the MHS exponent for cellulose in 0.5% LiCl/DMAc is in the order of 0.65.

$$[\eta] = K \cdot M^{0.65} \qquad (4.3)$$

Considering that McCormick et al. [30] found that $a = 1.19$ for cellulose in 8% LiCl/ DMAc this is a surprisingly low value. It was, however, shown in the same work that the intrinsic viscosity of a cellulose sample in LiCl/DMAc is strongly dependent on the LiCl concentration and decreases with decreasing LiCl concentration. This indicates that the cellulose molecule adopts a less extended conformation at low LiCl concentrations. The good correspondence between the data for different sulfite pulps (Figure 4.6) and the data for cotton linters cellulose (Figure 2b in **Paper II**) supports this results.

Although the low value of α of 0.55 and an estimated value of the MHS exponent a of 0.65 can be interpreted as an indication of cellulose being a random coil under these conditions, the value for the MHS exponent for cellulose in CED solution of 0.905 [57] and correspondingly high a values for cellulose derivatives like CMC [58, 59], indicate that the cellulose molecule does not necessarily adopt a random coil configuration under the prevailing conditions. Segment-segment interactions (intermolecular association) may also significantly decrease the hydrodynamic volume of the cellulose molecule and give α and a values closer to those which would be expected for a more flexible chain. Therefore, one cannot safely conclude that cellulose adopts a random coil configuration in 0.5% LiCl/DMAc. It seems, however, that the cellulose molecule has a significantly less extended chain configuration under these conditions than has been determined under other conditions.

4.3 Characterization of carboxymethyl cellulose

The molecular weight characterization of cellulose described in Chapter 4.2 has made it possible to study the influence of different raw material and carboxymethylation methods on the properties of the CMC product. Generally, to understand the macroscopic behaviour of carboxymethyl cellulose, such as a water binder, thickener and an emulsion stabilizer, a precise knowledge of both the chemical structure and molecular conformation is necessary. The chemical structure was determined by using ^{1}H and ^{13}C NMR spectroscopy and the molecular conformation was measured by size exclusion chromatography coupled with low angle laser light-scattering (LALLS) and differential refractometry detector (RI).

4.3.1 General considerations of chemical modification of cellulose

Due to the initial insolubility of cellulose, the chemical modifications are generally conducted under heterogeneous conditions. This results in a heterogeneous distribution of substituents along the polymeric chain. A blockwise distribution of the substituents and corresponding irregular properties are obtained which may give a fraction of gel-like material on dissolution. For these reasons, different methods of preparing cellulosic derivatives under heterogeneous and homogeneous conditions are used in order to obtain

products with homogeneous chemical structures and thus have properties that are independent of the origin of the initial cellulose.

4.3.2 Dissolution of CMC

All commercial grades of CMC consist predominantly of the Na salt of CMC and not of the free acid (H-CMC). The water solubility of CMC depends primarily on the DS, but is also influenced by the procedure of production and the DP. A macroscopic, clear solution at 1% usually contains (besides single macromolecules) temporary chain aggregates held together by hydrogen bonds and larger gel particles persisting from the raw material. As demonstrated in Figure 4.17 this part decreases with increasing DS. But it will also be influenced by the raw material and on the procedure of CMC preparation via differences in substitution patterns along the chains. This ill-defined structural state of aqueous CMC solutions makes the [η]-M relationships so far reported somewhat questionable.

Besides this fact, some difficulties are faced in the MWD analysis of CMC by SEC because of the polyelectrolyte properties of this polymer. Much effort has been made to suppress the polyelectrolyte expansion in solution [58, 60]. An acetate buffer or phosphate buffer is usually added to the eluent in order to reduce theses effects [58, 61]. The secondary polyelectrolyte effects such as ion exclusion may be reduced by varying the ionic strength and/or pH of the mobile phase [62, 63] and by using different types of columns [61, 64]. The apparent viscosity of aqueous solutions decreases significantly with increasing the content of low molecular electrolytes. In dependence on pH the apparent viscosity passes a maximum at pH~7. Influence of sample concentration, salt concentration and pH on the solubility of CMC is described in the literature [65].

In the present work the applicability of 0.05M Na_2SO_4/0.01M EDTA as a eluent for CMC has been investigated. This eluent has been used in the SEC-LALLS analysis of several polysaccharides e.g. β-glucans [66].

4.3.3 MW separation of CMC samples by SEC-LALLS

Figure 4.7 shows elution curves and corresponding calibration plots of three commercial CMC samples with different intrinsic viscosity (LV = low viscosity, MV = medium viscosity and HV = high viscosity). The samples eluted in accordance with their $\overline{M}_w$ and were well separated as demonstrated by the linear decrease in log M with increasing elution volume (Figure 4.7). The calibration plot is linear and overlapping over the molecular weight range $1.0 \cdot 10^3$ - $1.0 \cdot 10^5$. According to the LALLS signal no aggregation was observed for these three samples. However, a few exceptions of aggregation was observed for some other samples investigated, but this will be mentioned later. The effect of sample concentration was absent under the same conditions and was tested for the HV sample by injecting half concentration of the initial sample (1.85 mg/ml).

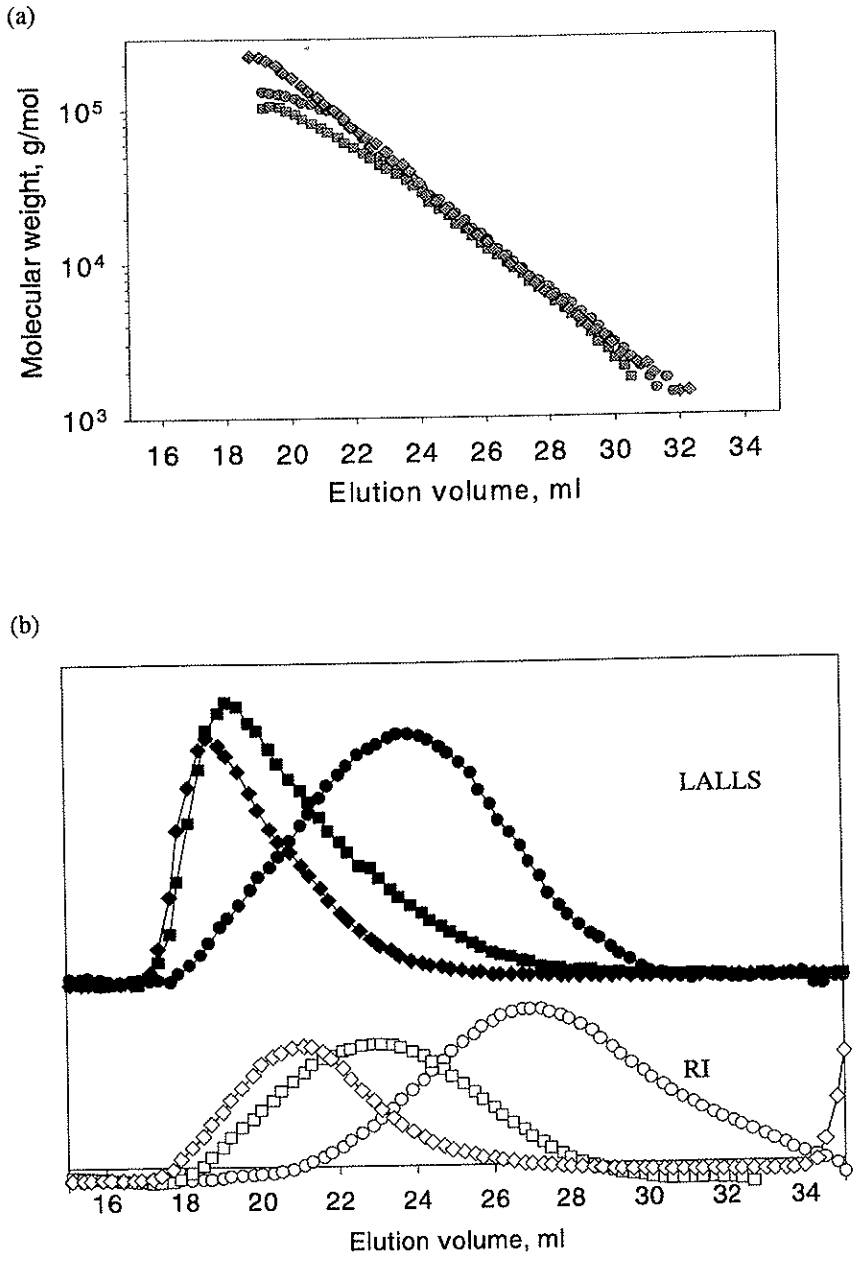

Figure 4.7 (a) Calibration plot, (b) Elution curves (RI signal) and light scattering (LALLS) signal of three commercial CMC samples. ◆: HV, ■: MV and ●: LV

4.3.4 Influence of MW and MWD of the initial cellulose on the CMC samples made by the heterogeneous carboxymethylation process

The CMC samples presented in Table 4.4 are produced by the two-phase isopropanol-water-NaOH system used in the heterogeneous carboxymethylation (conventional slurry ethertification process) and have a DS of 0.95 ± 0.10. The $\overline{DP}$ values of cellulose and CMC samples have been calculated on the basis of the $\overline{M}_w$ values obtained from the SEC-LALLS and SEC-MALLS data. The MW of each AGU of cellulose and Na-CMC was considered to be 180 and 243 (DS = 1.0), respectively.

The heterogeneous carboxymethylation caused a decrease in $\overline{DP}$ which was estimated to be ~35% in average for all samples. It is likely to believe that the chain degradation is caused by alkaline and radical reactions. However, the degree of degradation is constant and independent of the $\overline{M}_w$ to the initial raw material. Table 4.4 shows that the polydispersity indexes have increased by the carboxymethylation. This may be explained by an increasing amount of short chains as a result of alkaline peeling reactions.

The crystallinity indexes of the cellulose raw materials (acid sulfite pulps) are practical identical (Table 4.7). This means that all the samples are equally accessible for random distribution of carboxymethyl groups (DS ~ 1 for all samples) and for degradation reactions. The only difference between the samples is initial average molecular weight.

The elution profiles of CMC samples shown in Figure 4.8. indicates an increase in MW from sample D1 to D7. The LALLS signal of D3 showed very small amount of aggregates, while sample D4 showed enough aggregates to affect the $\overline{M}_w$ to an increased value.

Table 4.4: Comparison of DP and polydispersity index ($\overline{M}_w/\overline{M}_n$) of initial cellulose samples and comparable CMC samples with DS ~ 1

Sample	Intrinsic viscosity	Cellulose[a]		Carboxymethyl cellulose[b]	
	$[\eta]_{SCAN}$ (ml/g)	$\overline{DP}_w$	$\overline{M}_w/\overline{M}_n$	$\overline{DP}_w$	$\overline{M}_w/\overline{M}_n$
D1	380	1333	3.0	864	3.6
D2	520	2222	5.4	1461	5.8
D3	570	2500	4.6	1951	6.9
D4	750	3833	5.5	2198	6.4
D5	1200	5822	5.4	3683	6.0
D7	>1450	8056	4.9	5268	5.4

a. $(MW/AGU)_{Cellulose}$: 180

b. $(MW/AGU)_{Na\text{-}CMC}$: 243

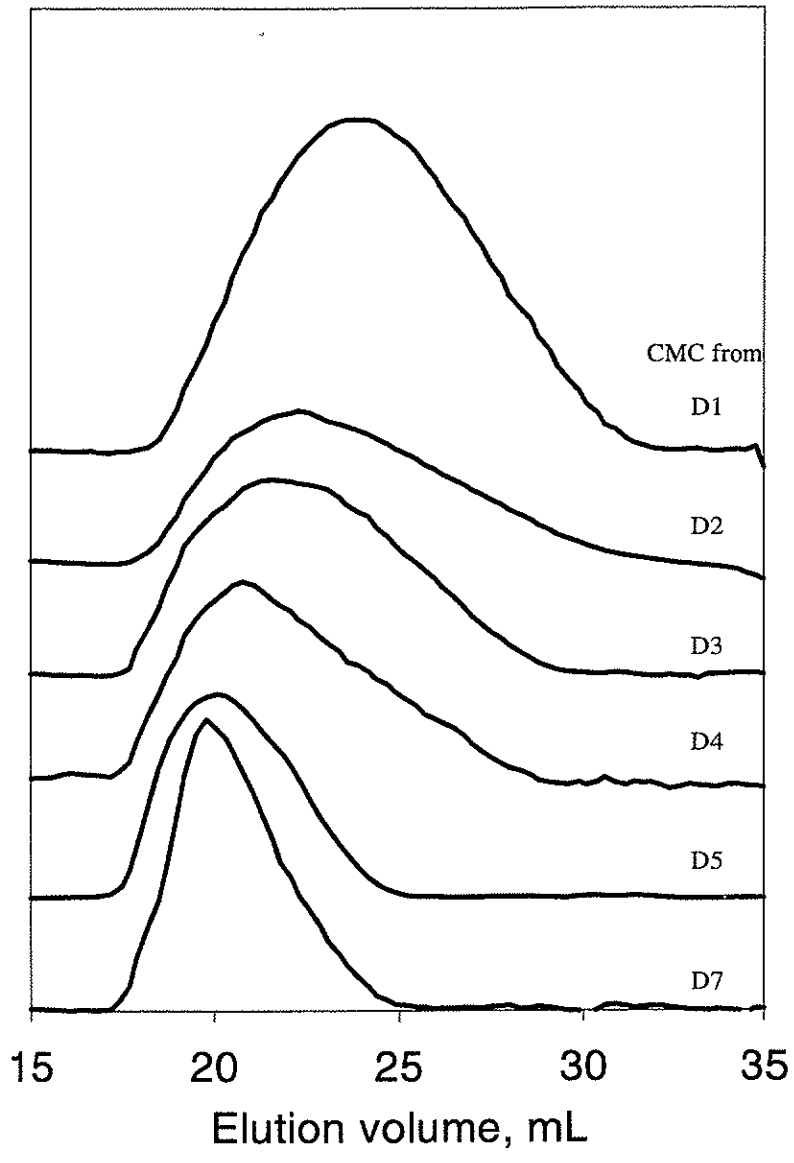

Figure 4.8 Elution curves of heterogeneously carboxymethylated cellulose samples (D1-D7) with DS~1.

4.3.5 Difference between heterogeneous and homogeneous carboxymethylation routes

Heterogeneously and homogeneously carboxymethylated CMC samples was made of a low (D3) and a high (D5) molecular weight cellulose sample. The average molecular weight ($\overline{M}_w$) was measured for the samples and are listed in Table 4.5. It is shown that the carboxymethylation leads to a decrease in molecular weight ($\overline{M}_w$) for both the heterogeneous and homogeneous methods. However, the chain degradation is more pronounced in the homogeneous carboxymethylation. By double carboxymethylation a higher degree of substitution is achieved through an additional amount of NaOH which also gives a further decrease in molecular weight.

Table 4.5: Molecular weight ($\overline{M}_w$) measured for D3 and D5

	Heterogeneous carboxymethylation		Homogeneous carboxymethylation	
Sample	Single	Double	Single	Double
	$\overline{M}_w$ ($\cdot 10^3$)	$\overline{M}_w$ ($\cdot 10^3$)	$\overline{M}_w$ ($\cdot 10^3$)	$\overline{M}_w$ ($\cdot 10^3$)
D3	474	358	289	217
D5	895	644	582	486

Figure 4.9 and Figure 4.10 shows the MWD of heterogeneously and homogeneously carboxymethylated CMC samples made of a low (D3) and a high (D5) molecular weight cellulose sample, respectively. In addition, elution curves of double carboxymethylated samples are shown.

Heterogeneously prepared CMC is produced in alkaline medium which swells the cellulose. The structure is opened up and the diffusion of reagent is increased. Degradation of cellulose under carboxymethylation in this environment is thought to proceed by alkaline hydrolysis or by radical reactions.

For the homogeneously carboxymethylated samples it is observed that the reaction leads to bimodal elution curves (Figure 4.9 and 4.10). The bimodal distribution is even more pronounced by the double homogeneously carboxymethylated samples. This indicates that a non-random degradation is taking place. Two possible explanation may be given for this:

The synthesis of CMC is a nucleophilic substitution. When a hydroxy group on a AGU in cellulose have been substituted the AGU is becoming more electrophilic and acidic [67]. The AGU is then more exposed to a nucleophilic attack. This means that the reaction goes more easily on a already substituted unit. At the same time the unit becomes more exposed to degradation. This should also be observed in the heterogeneous made CMC samples. When a bimodal distribution is not observed for the heterogeneous system it is likely to believe that this explanation fails.

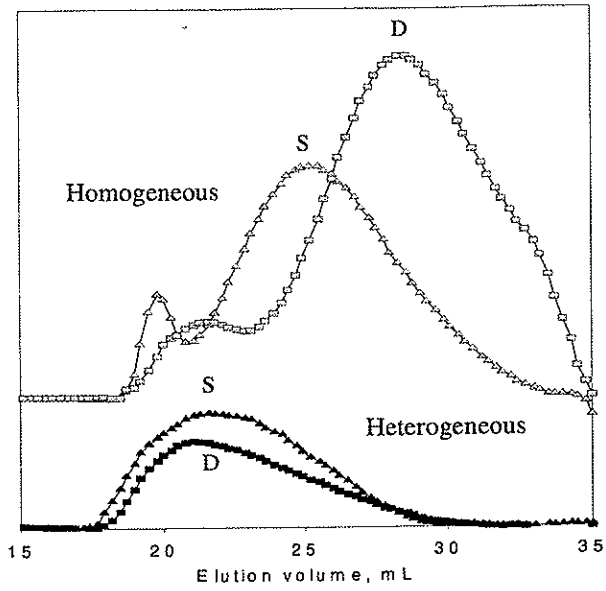

Figure 4.9 Elution curves of heterogeneous (black) and homogeneous (grey) carboxymethylated cellulose of D3. S = Single carboxymethylation, D = Double carboxymethylation.

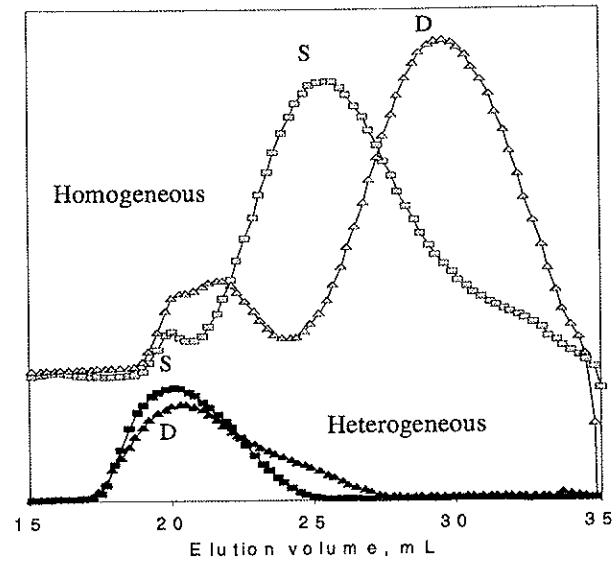

Figure 4.10 Elution curves of heterogeneous (black) and homogeneous (grey) carboxymethylated cellulose of D5. S = Single carboxymethylation, D = Double carboxymethyaltion.

Topologic effects may lead to that some molecules are more exposed to degradation than others. This may be caused by one or a combination of the three following effects: Firstly, an internal association of cellulose may protect molecules against degradation. This pre-requests a deposition of substituted CMC to some extent. Secondly, if the degradation occurs immediately when the reagents is added, the cellulose molecule may be damaged before the substitution reaction starts. Possible aggregates are protected against substitution. Thirdly, if the reagents is not soluble in LiCl/DMAc this results in a two-phase system. A carboxymethylation may then give rise to a bimodal distribution provided that the rate of diffusion is the rate limiting factor of the reaction.

4.3.6 Heterogeneous-II carboxymethylation

Elution curves of the third carboxymethylation method (heterogeneous-II) is shown in Figure 4.11. Three CMC samples (1, 2 and 3) were produced by different molar ratios of the reagents (Table 4.6). The degree of polymerization ($\overline{DP}_w$) of the produced CMC is approximately 25% of initial acid sulfite pulp, $\overline{DP}_w$(D3) = 2500. Generally, a considerable chain degradation has taken place during the carboxymethylation. The pulp sample have been exposed to a strong swelling by Me_2SO and Me_4U before addition of the reagent. This have made the pulp more easily accessible towards the reagents and thereby more exposed to chain degradation by alkali during the derivatization process.

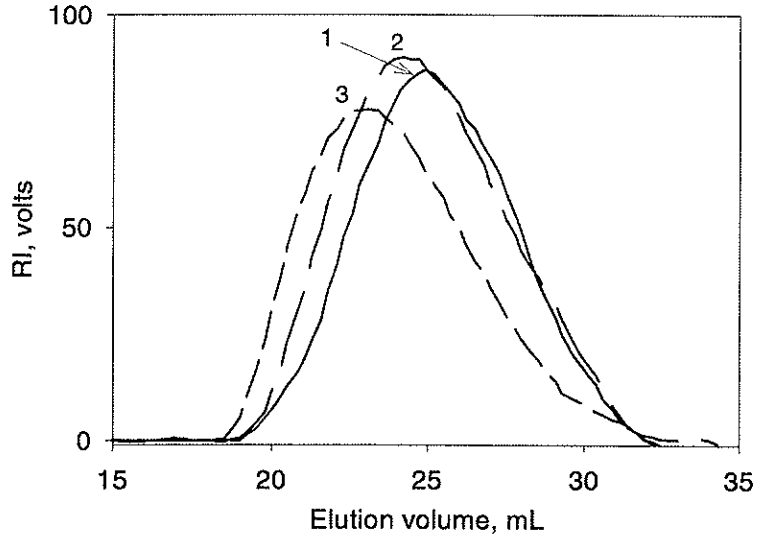

Figure 4.11 Elution curves of three heterogeneously (heterogeneous-II) carboxymethylated samples of acid sulfite cellulose sample D3. Table 4.6 shows the resulting $\overline{M}_w$ of the CMC samples 1, 2 and 3.

Table 4.6: The effect of molar ratio of the reagents on the $\overline{M}_w$ of the CMC samples originated from the acid sulfite pulp D3

Sample	NaOH (mol)	MCA (mol)	DS	$\overline{M}_w$ ($\cdot 10^3$)	M_0	$\overline{DP}_w$
1	2	2	2.0	200	324.0	617
2	4	4	2.2	226	340.5	664
3	8	8	2.4	240	356.5	674

The molecular weight ($\overline{M}_w$) is increasing with increasing amount of reagent added even though small changes is observed (Table 4.6). Although the reaction is conducted under nitrogen atmosphere it is experimentally difficult to exclude all oxygen. As long as the reaction has access to oxygen, a radical degradation may proceed. Acetic acid is working as a radical scavanger [68, 69], and it is shown that acetic acid has a preventing effect on carbohydrate degradation through radical reactions in ozone bleaching [70]. It is possible that MCA has the same effect. If this is true a decreased radical degradation may be observed by increasing the addition of MCA.

4.4 Cellulosic structure during carboxymethylation

Chemical and physical properties of cellulose ethers like carboxymethylcellulose are determined not only by the average substitution, but also by the positions of the substituents at the anhydroglucose units and by the distribution of the subsitutents along the cellulose chains. The structure of cellulose may influence the substitution and substitution pattern. Therefore it is interesting to characterize the structure of cellulose raw material.

4.4.1 Crystallinity measurements of initial pulps

The cellulose molecule differs in regions which are completely ordered (such as those found in the interior of crystallites) through those which are amorphous, or lacking any long range order. Methods for measuring the crystallinity have different sensitivity to distinguish between crystalline and less ordered regions. Chapter 2.4.6 briefly summarize how NMR spectroscopy and X-ray diffraction distinguishes between crystalline and less ordered regions [71].

The crystallinity index of the initial cellulose material was measured by NMR and X-ray diffraction in this study. The results are listed in Table 4.7 and shows that there are only minor differences in the crystallinity of the initial cellulose samples before carboxymethylation. Comparing the two methods of determining the crystallinity shows a systematic difference for spruce sulfite celluloses measured by NMR compared to X-ray.

The same agreement has not been obtained for the cotton linter cellulose (CLC). This could be explained when considering that the two methods are sensitive to different aspects of ordered and disordered cellulose or due to the limited number of measurements. X-ray diffraction underestimates the crystallinity index if a large amount of small crystallinity areas is present [71]. If this is the case for the cotton linter cellulose sample it could explain why the value of crystallinity is higher as measured by NMR than measured by X-ray diffraction.

Table 4.7: Crystallinity of initial cellulose

Sample name	NMR (%)	X-ray (%)
D1	51.0	-
D2	50.0	-
D3	52	57.8
D4	53.0	-
D5	53.5	55.9
D7	50.0	-
C3	61.0	54.7

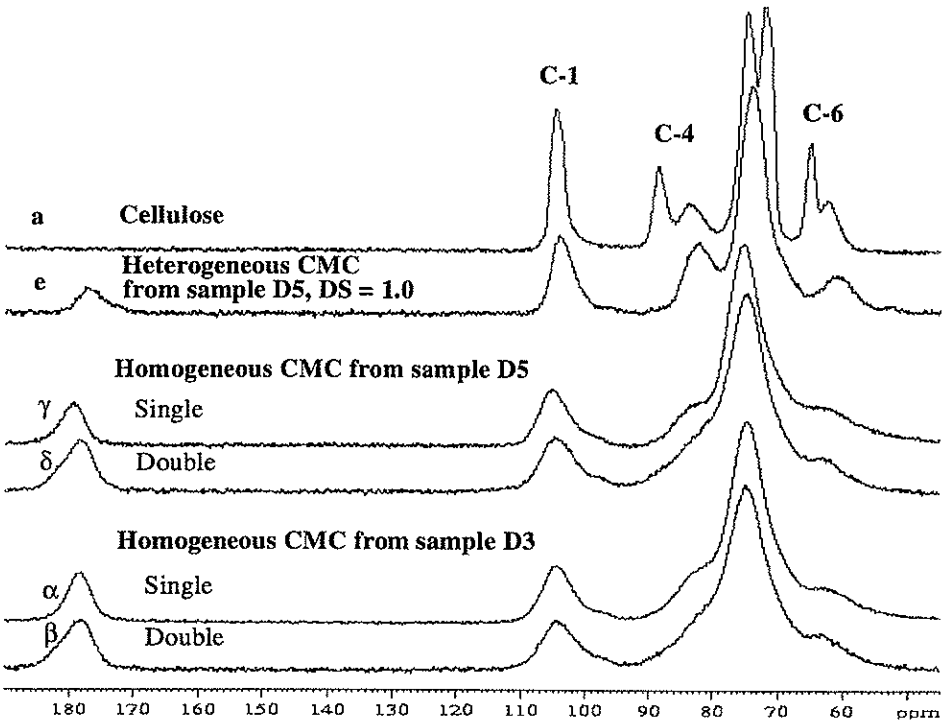

Figure 4.12 ^{13}C CP-MAS spectrum of heterogeneously and homogeneously prepared CMC from acid sulfite pulp cellulose. (a) acid sulfite pulp cellulose sample, (e) reference CMC sample (without MCA), (α–β) CMC of D3, and (γ–δ) CMC of D5.

4.4.2 Comparison of the heterogeneous and homogeneous method

Sample description and conditions for the carboxymethylation reactions presented are listed in Table 4.8.

Table 4.8: Conditions and results of the heterogeneous and homogeneous carboxymethylation of acid sulfite pulps.

Starting material	Type of reaction	Specter name of CMC	NaOH (g)	Molar ratio MCA/AGU	Reaction time (h)	DS
D5	hetero	**b**	5	0	0	0
		c	5	1.5	2.5	0.5
		d	5	1.5	2.5	0.8
		e	10	2	4	1.0
	double hetero	**f**	5	2	4	1.5
D3	hetero	**h**	5	0	0	0
		i	5	1.5	2.5	0.5
		j	5	1.5	2.5	0.8
		k	10	2	4	1.0
	double hetero	**l**	5	2	4	1.5
D5	homo	α	5	5	4	1.8
D3		β	5	5	4	1.8
D5	double homo	γ	5	5	4	2.2
D3		δ	5	5	4	2.2

Figure 4.12 shows the ^{13}C solid state NMR spectra of heterogeneously (**e**) and homogeneously (α–δ) carboxymethylated cellulose, which demonstrate differences between the two derivatization methods. For comparison, the NMR spectrum of the initial acid sulfite pulp cellulose (**a**) is shown in the upper part of Figure 4.12. The lines are assigned to the carbon atoms of the AGU, where the narrow and wide components of the C-4 and C-6 peaks represent the crystalline and non-crystalline regions of the sample, respectively. Comparing sample D5 and D3 in the figure to the spectrum **a**, one can see, above all, the change in the intensity of the narrow parts of the lines C-4 and C-6 at 89 and 66 ppm, which are characteristic for ordered cellulose I [72]. From their absence in the spectra of heterogeneous and homogeneous CMC, it can be concluded that in both reactions, the entire initial cellulose is made accessible to the reaction with MCA. There are only minor changes in the NMR spectra of singly and doubly (spectrum β and δ) carboxymethylated samples. It is published by Mann et al. that primarily the non-crystalline regions are derivatized in selective carboxymethylation (synthesized by the reversed method [73]) [74]. This is seen by the remaining crystalline C-4 and C-6 line components in the spectrum.

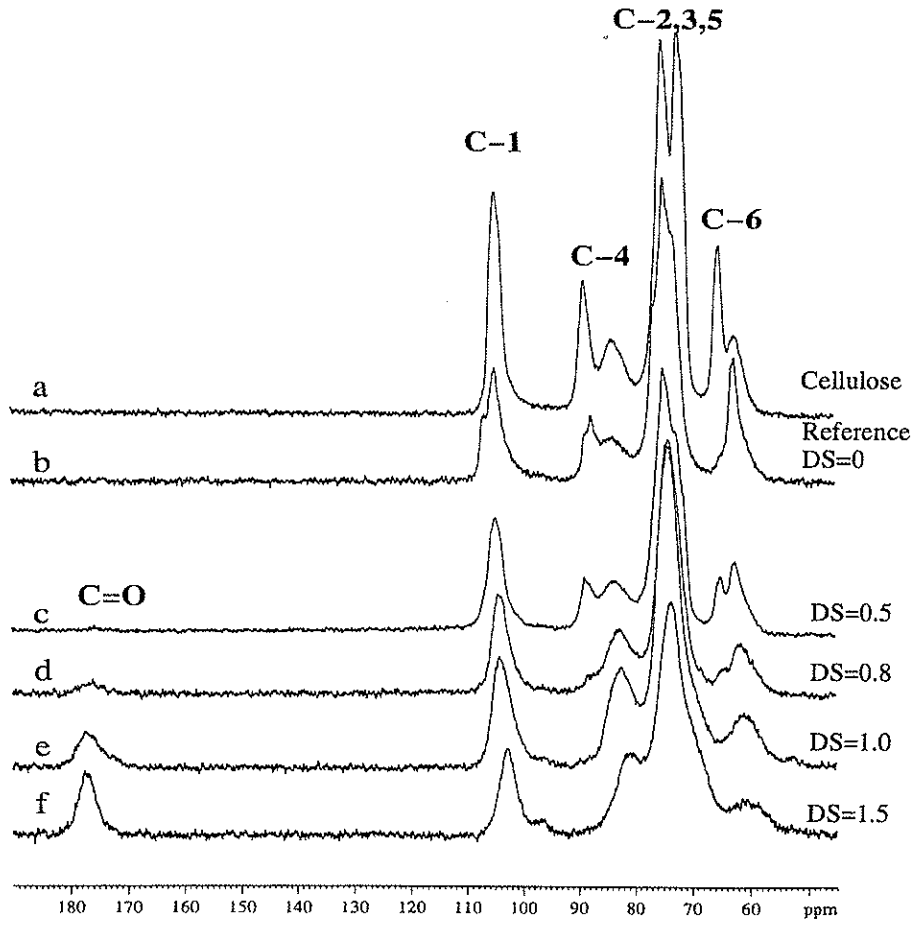

Figure 4.13 50MHz ^{13}C CP-MAS NMR spectra of cellulose and CMC samples of varying DS before dissolution. The cellulose raw material was a acid sulfite pulp of η_{SCAN}=1200 ml/g.

4.4.3 Heterogeneously synthesized CMC with different degree of substitution

Figure 4.13 shows the solid state NMR spectra of CMC originated from heterogeneously carboxymethylated D5 (high viscosity). The spectra differ only in the degree of substitution (0.5-1.5). Spectrum **a** and **b** in the figure represents the initial cellulose and reference CMC sample respectively. The reference CMC sample of a spruce sulfite cellulose sample is alkalized but without addition of the etherification reagent (MCA). This leads to a structural conversion of the ordered native cellulose I into alkali-cellulose using concentrated solutions of NaOH.

The gradual change in the intensity and area of the narrow parts of the lines C-4 and C-6 at 89 and 66 ppm clearly show a decreasing amount of ordered cellulose with increasing DS. Low-substituted CMC's manufactured by the heterogeneous reaction method seem to retain a significant amount of crystalline cellulose. This seems to be mainly present in the insoluble fraction. A DS significantly above unity could be reached by performing a second heterogeneous carboxymethylation. This lead to a loss of the crystalline structure.

By increasing the NaOH concentration it is likely to believe that the heterogeneous carboxymethylation hardly occurs in the ordered structural regions (difference between specter **c** to **d,** Figure 4.13) [74]. This means that the entire initial cellulose is gradually been made accessible to the reaction with MCA and more of the crystalline regions of the cellulose is included in the reaction. From the lineshape of C-4 (Figure 4.13) it appears that cellulose I crystallites are still present [75] in the CMC up to DS = 0.8, but only insignificant amounts are detectable above this value [76].

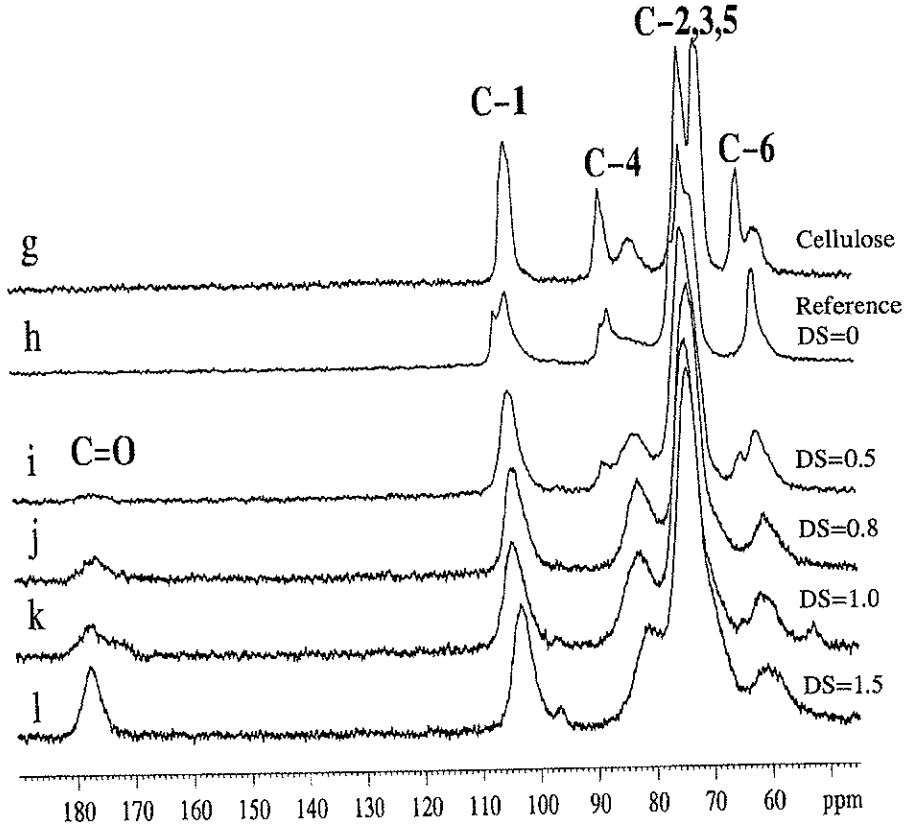

Figure 4.14 50MHz ^{13}C CP-MAS NMR spectra of cellulose and CMC samples of varying DS before dissolution. The cellulose raw material was a acid sulfite pulp of η_{SCAN}=570 ml/g.

Figure 4.14 shows ^{13}C CP-MAS NMR spectra of a low viscosity cellulose (D3) and CMC samples of varying DS. In comparison with the above discussed sample, D5 (Figure 4.13), the NMR result shows a lower crystallinity for the low-substituted CMC (**i** versus **c**). However, the fraction of cellulose II seems to be higher for D3 (Figure 4.14)[77].

In addition to NMR, sample D3 and D5 have been analyzed by X-ray diffraction. Diffractograms of the initial cellulose of sample D5 and corresponding CMC are shown in Figure 4.15. The diffractogram of the crystalline component was refined by averaging the estimates of the crystalline components. This method gives only relative values due to the lack of a 100% crystalline cellulose standard. The X-ray results indicate that essentially all the crystalline cellulose present in the spruce sulfite cellulose is lost during the heterogeneous carboxymethylation. Only minor changes in crystallinity have been measured for CMC with different DS. It is clearly shown that the main crystallinity part is lost during the alkalization to Na-Cellulose and that only minor crystallinity changes occurs when increasing the MCA/AGU ratio. Reaction time seems to be an important factor for decreasing the crystallinity further in addition to stepwise addition of NaOH [74].

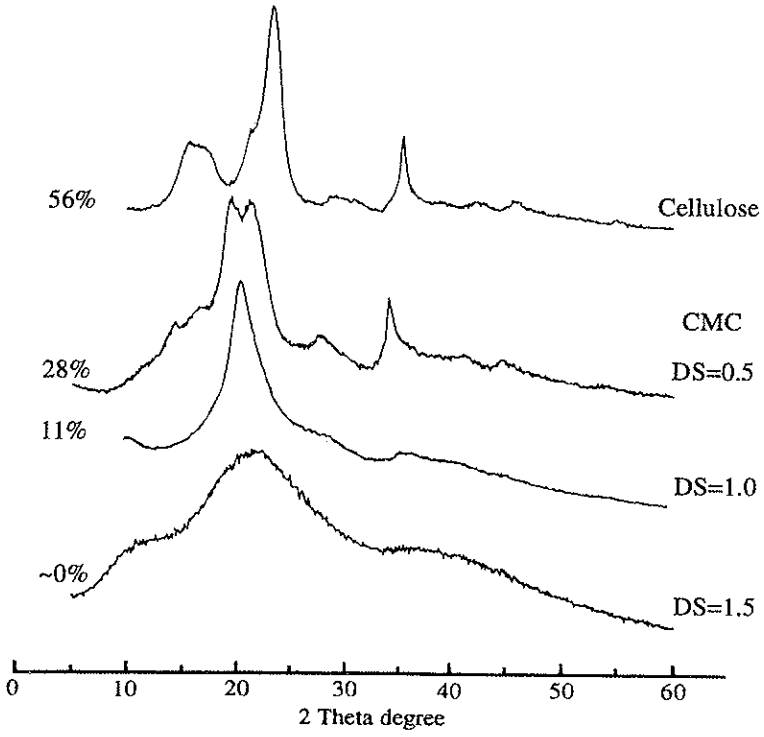

Figure 4.15 X-ray diffractograms and crystallinity index of cellulose and CMC samples of varying DS before dissolution.The cellulose raw material was D5 (1200 ml/g).

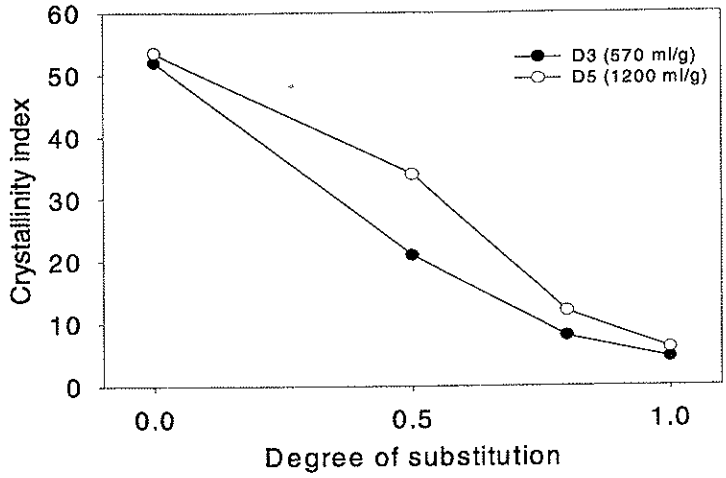

Figure 4.16 Crystallinity index of CMC samples of varying DS as determined by ^{13}C CP-MAS NMR spectroscopy. DS=0 is reference cellulose which has been alkali treated.

The NMR data show that cellulose samples with similar degree of crystallinity but different molecular weight, gave (after carboxymethylation) different degrees of crystallinity (Figure 4.16) and different amounts of insoluble fraction (Figure 4.17).

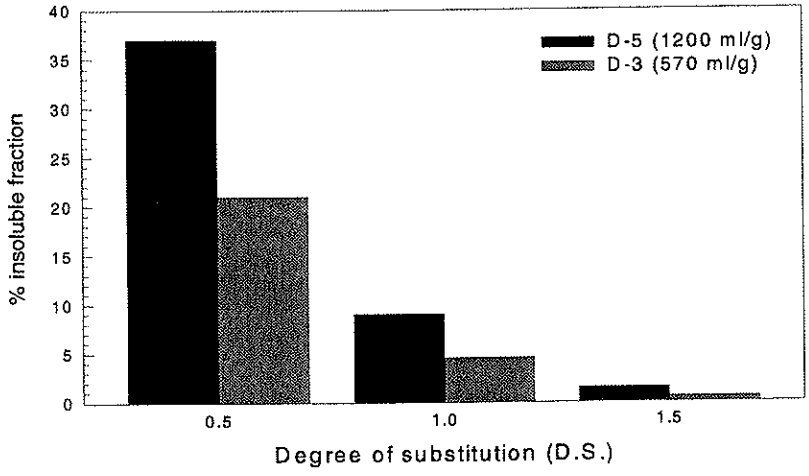

Figure 4.17 Insoluble fraction of CMC samples of varying DS. The insoluble fraction was determined gravimetrically after filtration.

4.4.4 Characterization of the insoluble fraction of CMC

Solution properties of CMC are controlled by varying the uniformity of substitution, the DS and the degree of polymerization (DP). In general, low-substituted types (DS=0.3 or less) are insoluble in water but soluble in alkali. Block-like distribution of the substituents of selectively carboxymethylated cellulose is obtained in dissolving experiments [77]. Heterogeneously synthesized CMC is either completely soluble in water, or it gives soluble and insoluble parts. Under the same conditions samples of homogeneously carboxymethylated cellulose exhibit only more or less gel-like particles detectable by microscopic investigations. From a theoretical point of view, the case of numerous unsubstituted crystalline network junctions may prevent the transition into the solution state.

The relation between amount of insoluble fraction and DS is shown in Figure 4.17 for sample D3 and D5. Carboxymethylation of acid sulfite pulp by the heterogeneous reaction method yields a substantial fraction of insoluble CMC with a very low DS. The insoluble fraction decreases with increasing DS. Cellulose samples with a similar degree of crystallinity but different molecular weight gave, after carboxymethylation, different degrees of crystallinity and different amounts of insoluble fraction. The fraction of insoluble CMC is higher for high molecular weight samples.

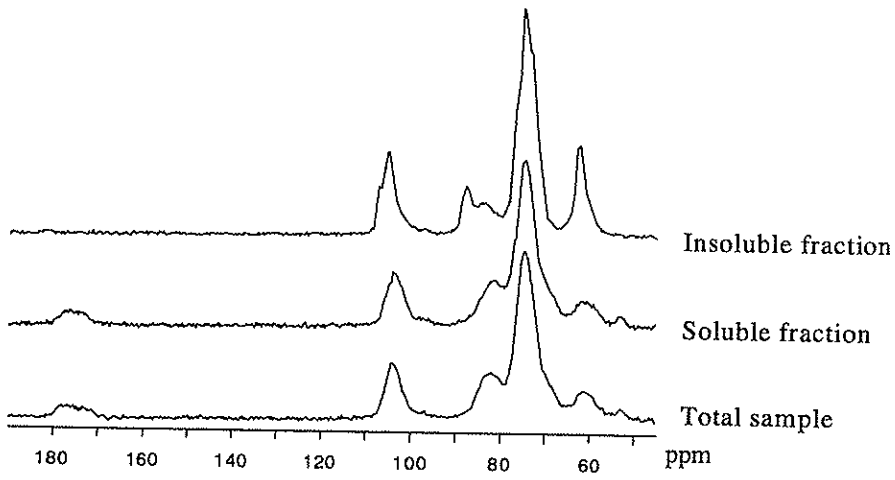

Figure 4.18 ^{13}C CP-MAS spectrum of insoluble and soluble fraction after filtration.

The NMR spectra in Figure 4.18 shows that the insoluble part of CMC, after filtration through a 0.3 μm filter, mainly consists of unsubstituted cellulose. The DS has been estimated to 0.15 in the sample. This means that on average only 1 per 6.7 AGU have one carboxymethyl group attached. The insoluble part of CMC probably originates from the crystalline areas which have been less substituted than the amorphous areas according to accessibility. By comparing the spectrum with that obtained of reference CMC (Figure 4.13, specter **b**) it can be concluded that the cellulose mainly consists in the crystalline state as cellulose II. However, the lines assigned to C-4 and C-1 are splitted.

This indicates that both the cellulose I and the cellulose II polymorphs may be present in the insoluble fraction.

The results from X-ray diffraction shows that the crystallinity index is very low for the CMC sample D5 with DS = 1. The crystallinity index was estimated to approximately 11% for the sample which has not been diluted in water. After dissolution this has decreased to 6%. This means that the structure of heterogeneously synthesized CMC is changed after dissolution in water. The crystallinity index for the insoluble and soluble part was estimated to 9% and ~0%, respectively.

CHAPTER

5

CONCLUDING REMARKS

Molecular characterization of cellulose by SEC has on several occasions been limited by plugging and adsorption effects on the columns in addition to the well-known problems of incomplete dissolution and aggregation of cellulose. A new alternative column matrix material, macroporous monodisperse particles (MMP), minimized the problems which may be encountered in SEC analysis of cellulose samples. Compared to the commercial column PLgel Mixed B it was found that MMP allowed better chromatographic stability both with respect to back pressure and reproducibility. A high sample recovery indicated that no significant adsorption of cellulose to the column material is taking place in this system. A modified dissolution procedure was developed for acid sulfite pulp samples. The MALLS data show no sign of aggregates present in the solution of cellulose and no adsorption to the column is observed. The average molecular weight ($\overline{M}_w$) obtained from SEC-MALLS correlates well with $\overline{M}_v$ calculated from intrinsic viscosity (measured by the SCAN procedure).

By this method of analyzing the MW and MWD of cellulose, it thus is now possible to characterize differences between cellulose samples. It also forms the basis for investigating the changes in MW and MWD of cellulose derivatives produced caused by different reaction methods.

During the acid sulfite cooking the cellulose molecules will be subjected to a non-random hydrolytic attack. For this reason, and because of the inhomogeneous fiber morphology of softwoods, a broad molecular weight distribution can be expected and is actally demonstrated in this work. When increasing the cooking time and temperature one can notice a shift in average molecular weight to lower values and a slight trend towards a narrower MWD is observed.

In this work the commercially most important cellulose ether, carboxymethyl cellulose, was used to study the molecular changes during cellulose derivatization. The CMC samples were dissolved in 0.05M Na_2SO_4/0.01M EDTA. Depending on the carboxymethylation method different MW and MWD values were obtained. A general

decrease in molecular weight ($\overline{M}_w$) was observed during carboxymethylation. The chain degradation increased by increasing amount of reagent and degree of substitution, and was independent of initial molecular weight. An exception was however observed for the heterogeneous-II carboxymethylation method. Homogeneous carboxymethylation caused a bimodal molecular weight distribution of CMC which indicates the presence of a non-random degradation.

The structure of cellulose may influence the degree of substitution and the substitution pattern in cellulose derivatives. It was therefore interesting to characterize the structure of cellulose raw material. The molecular structure of acid sulfite cellulose and CMC was studied by SEC-MALLS, NMR spectroscopy and X-ray diffraction. By the R_G-M relation, MALLS data showed that cellulose can be considered to be a random coil in 0.5 % LiCl/DMAc. On the basis of the same data, the exponent, *a*, in the MHS equation was estimated to 0.65.

Carboxymethylation of spruce sulfite cellulose by the heterogeneous reaction method yields a substantial fraction of insoluble carboxymethyl cellulose (CMC) with a very low degree of substitution and a high degree of crystallinity (measured by NMR and X-ray). There are indications that both the cellulose I and the cellulose II polymorphs are present in the insoluble fraction. Cellulose samples with a similar degree of crystallinity however different molecular weight gave, after carboxymethylation, different degrees of crystallinity and varying insoluble fraction. Low-substituted CMC's produced using the heterogeneous reaction method seem to retain a significant amount of crystalline cellulose. This appears to be present mainly in the insoluble fraction. A DS significantly above unity could be reached by undertaking a second heterogeneous carboxymethylation. This leads to a loss of the crystalline structure. Although the crystallinity indices of the cellulose samples were fairly similar, the high molecular weight spruce sulfite cellulose sample retained more of its crystalline structure at low degrees of substitution.

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APPENDIX

A.1 Chemicals

Table A.1: List of chemicals

Chemical	Grade	Company
Acetone	p.a.	Acros
Methanol	p.a.	Sigma-Aldrich
N,N-dimethylacetamice (DMAc)	p.a.	Burdick & Jackson
Lithium chloride (LiCl)	98%	Baker
Ethylene diamine tetraacetic acid (EDTA)	99%	Fluka
Diethylene triamine pentaacetic acid (DTPA)	99%	Fluka
Citric acid	p.a.	Merck
NaOH	p.a.	Kebo
Monochloro acetic acid (MCA)	98%	Riedel-deHaën
Isopropanol	98%	Arcus
1,1,3,3-tetramethyl urea	p.a.	Merck
Dimethyl sulfoxide (Me_2SO el. DMSO)	p.a.	Merck
Ethanol (EtOH)	96%	Arcus
Calsium chloride	96%	Merck
Molecular sieve, 4Å	-	Merck
Sodium sulfat (Na_2SO_4)	>99%	Merck
Cupriethylenediamine (CED)	Cu 1.0 ± 0.02M	Borregaard Ind. Ltd.
Perchloric acid	p.a.	Merck
Sulfuric acid	p.a.	Merck
Trifluoroacetic acid (TFA)	99%	Acros
Deuterium oxide	99.9%	Fluorochem Ltd.
3-(trimethylsilyl)-propionic acid-2,2,3,3-d_4 sodium salt (TSP)	min. 98% deuterium degree, for NMR spectroscopy	Merck
Hexamethyldisiloxan (HMDSO)	>99.5%, for NMR spectroscopy	Fluka
Carboxymethyl cellulose (CMC)	LV	Sigma
CMC	MV	Sigma
CMC	HV	Sigma
Silver nitrate	p.a.	Merck

A.2 Polystyrene standards

Table A.1: Polystyrene standards obtained from Polymer Laboratories.

Sample name	$\overline{M}_w$ (g/mol)
PS1	4,000
PS2	20,650
PS3	28,500
PS4	200,000
PS5	400,000
PS6	900,000
PS7	2000,000

A.3 Cotton linter cellulose

Table A.2: Cotton linter samples

Sample name	SCAN viscosity number, dm^3/kg
C1	380
C2	1200
C3	1550
C4	1900

A.4 Acid sulfite pulp cellulose

Table A.3: Characterstics of different acid (spruce) sulfite pulps

Sample name	Borregaard grade	S18	S10	α-content	SCAN viscosity number	DCM	ISO brightness	Ash	Ca	Fe	Si	Mn
D1	Derivat LV-U	6.5	15	89	380	0.1	94	0.1	40	2	30	1
D2	Derivat LV	6	12	91	520	0.1	93	0.1	40	2	30	1
D3	Dissolving SVS-HA	4	9	93.5	570	0.1	93	0.1	40	2	30	1
D4	Derivat NC	6.8	11	91	750	0.1	93	0.05	40	2	40	1
D5	Derivat HV	6.8	9	92	1200	0.1	88	0.2	150	3	60	1
D6	Derivat VHV-HA	4.5	6	95	1350	0.1	88	0.2	100	3	60	1
D7	Derivat VHV-S	7.5	8.5	92	>1450	0.1	84	0.3	150	3	60	1
D8	Derivat VHV-U	7.5	8.5	92	>1550	0.1	82	0.3	150	3	60	1

Table A.4:

Characteristics	Method	Unit
Alkali solubility S18	SCAN C2:61	%
Alkali solubility S10	SCAN C2:61	%
CED-viscosity	SCAN CM15:88	dm^3/kg
Dichloromethane (DCM) extract	SCAN C7:62	%
ISO Brightness	SCAN C11:75	%
Ash	SCAN C6:62	%
Calcium	SCAN C10:62	mg/kg
Iron	SCAN CM13:87	mg/kg
Silicates and silica	SCAN C9:62	mg/kg
Manganese	SCAN CM14:87	mg/kg

α-calculated:

$$\alpha = 100 - \frac{(S18 + S10)}{2}$$

PAPER I

SIZE EXCLUSION CHROMATOGRAPHY OF CELLULOSE DISSOLVED IN LICL/DMAC USING MACROPOROUS MONODISPERSE POLY(STYRENE-*CO*-DIVINYLBENZENE) PARTICLES

TOVE SCHULT, STØRKER T. MOE*

NTNU Department of Chemical Engineering
Sem Sælands vei 4
N-7491 Trondheim
NORWAY

TORGEIR HJERDE

Borregaard ChemCell
P.O Box 162
N-1721 Sarpsborg
NORWAY

BJØRN E. CHRISTENSEN

Norwegian Biopolymer Laboratory (NOBIPOL)
NTNU Department of Biotechnology
Sem Sælands vei 6-8
N-7491 Trondheim
NORWAY

ABSTRACT

Two types of hydrophilic macroporous monodisperse particles (MMP) based on poly(styrene-*co*-divinylbenzene) have been investigated for use as column material for size exclusion chromatography (SEC) analysis of cellulose dissolved in lithium chloride/*N*,*N*-dimethylacetamide (LiCl/DMAc). The particles appeared inert to the mobile phase and no adsorption of cellulose could be detected. Favorable chromatographic properties associated with monosized particles such as low back pressures and high flow rates were obtained. Two MMP columns of different pore size distributions coupled in series allowed separation of cellulose in the molecular weight range 10^4-10^7 g/mole. The particles were suitable for SEC of cellulose samples dissolved in 0.5% LiCl/DMAc and the reproducibilty and long term stability were superior to that of a comparable commercial SEC column.

* Author for correspondence

INTRODUCTION

As probably the most abundant polymer in the world, cellulose is a compound of great industrial importance. Cellulose has a number of different applications both as a fiber, a chemical substance and as a raw material for the synthesis of cellulose derivatives. The molecular and supramolecular structure of cellulose seems to be well described in the literature, but due to its extremely low solubility in most known solvents, the analysis of some molecular properties such as molecular weight (MW) and molecular weight distribution (MWD) of high molecular weight celluloses is still not a trivial task.

Cellulose is a simple homopolymer of β-D-glycopyranose units linked by (1-4)-glycosidic bonds. The equatorial conformation of the glycosidic bond and the presence of only equatorial substituents on the glycosidic ring makes cellulose a polymer with a very large degree of stereoregularity. Crystalline structures stabilized by inter- and intramolecular hydrogen bonds are found in cellulose samples, and the dense crystalline structure and the high number of intramolecular hydrogen bonds makes cellulose virtually insoluble. However, a solvent for cellulose, lithium chloride/*N,N*-dimethylacetamide (LiCl/DMAc), has been described by McCormick [1]. This non-degrading solvent for cellulose has opened the possibility of size exclusion chromatography (SEC) analysis of cellulose [2-5]. However, in practice, cellulose has limited solubility in this solvent, especially at high molecular weights. Possible problems in SEC [6-9] may be eliminated by choosing more suitable column materials (presented here), while the former requires improvements in the procedure and conditions for the dissolution of cellulose (presented in another work [10]).

In this work we report on the application of a new type of macroporous, monodisperse polymer particles (MMP) for SEC analysis of cellulose in LiCl/DMAc. The particle matrix consists of highly crosslinked poly(styrene-*co*-divinylbenzene) with a hydrophilic layer covalently linked to the surface of the pores. To evaluate the performance of the investigated column material, one commercial column system was included in the present study. Similar MMP have been used for SEC analysis of polysaccharides in aqueous solutions [11,12]. In addition to extending the limiting molecular weight to about 10^8 g/mole for coil-like polysaccharides such as pullulans, and to about 10^7 g/mole for rod-like polysaccharides such as scleroglucans, favorable chromatographic properties generally associated with monosized particles (e.g. very low back pressures and fast separations) were reported [11].

EXPERIMENTAL

Materials

Polystyrene standards, designed PS1 - PS7, with weight average molecular weight (M_w) of 4000, 20650, 28500, $2.0 \cdot 10^5$, $4.0 \cdot 10^5$, $9.0 \cdot 10^5$, and $2.0 \cdot 10^6$ g/mole, respectively, were obtained from Polymer Laboratories. Three cellulose samples, MCC (microcrystalline cellulose), CLC (cotton linter cellulose) and SSC (spruce sulfite cellulose) were obtained from Borregaard Ind. Ltd., Peter Temming and Borregaard Ind. Ltd., respectively.

Materials used for dissolution of polystyrene and cellulose samples were *N,N*-dimethylacetamide (DMAc, p.a. grade, Burdick & Jackson) and methanol (p.a. grade, Sigma-Aldrich), both dried with molecular sieves (Type 4A, Merck), acetone (p.a. grade, Acros), ethylene diamine tetraacetic acid, (EDTA, 99%, Fluka), diethylene triamine pentaacetic acid (DTPA, 99%, Fluka), citric acid (p.a. grade, Merck), lithium chloride (98%, Baker, ovendried and stored at 150°C) and deionized water. The molecular sieve was activated by heating to 450°C for 3 days.

Column material

The macroporous monodisperse particles were produced by SINTEF Applied Chemistry (Trondheim, Norway). Two particle types (I and II), both with a diameter of 15μm, were prepared as described by Ellingsen *et al.* [13].

The pore size distribution and specific pore volume of the particles were measured by mercury porosimetry [14]. The specific surface area was measured by the Brunauer-Emmett-Teller (BET) method [15]. A commercial SEC column, PLgel 10μm Mixed B (7.5 x 300 mm, 3 in series) was obtained from Polymer Laboratories and was included in the study for comparison.

Column I was packed with particle I and column II was packed with particle II. Column II+I is the combination of column II and column I coupled in series.

Column packing was performed essentially as described by Christensen et al. [11]. An aqueous suspension of particles was washed with ethanol and packed in a vertically mounted column (Pharmacia HR 10/30, i.d. = 10 mm, l = 300 mm) at a flow rate of 1.0 ml/min. During

packing, the column was vibrated at 50 Hz. Solvent exchange was performed in the following order: 0.1 M NaCl, water, methanol, DMAc and finally 0.5% LiCl/ DMAc.

Sample preparation

The cellulose samples were swollen in deionized water (24 hours, room temperature) and subsequently washed with EDTA, DTPA, and finally citric acid [10]. The cellulose was further washed with 0.1M LiCl. An acetone extraction was then performed to remove possible extractives left in the pulp. The sample was solvent exchanged with methanol and DMAc before dissolution in 8% LiCl/DMAc. Before injection, the solution was diluted with DMAc to 0.5% LiCl/DMAc and filtered through a solvent resistant, disposable teflon filter (Millex SR 0.5μm for the SSC and MCC samples and Millex CN 3.0μm for the CLC sample, both filters from Millipore).

Size exclusion chromatography

SEC was performed at 40°C using a Perkin Elmer (Series 2000) HPLC pump with autosampler, operating at a flow rate of 1.0 ml/min. Injected samples contained 50-200 μg of dissolved cellulose (200 μl). The elution was monitored by a refractive index detector (Shimadzu RID-10A) and a multi-angle laser light scattering detector (MALLS, Wyatt Dawn DSP, 633 nm) equipped with an in-line filter holder (Millipore) with 0.2 μm PTFE-filter (Fluoropore-FG, Millipore). The refractive index increment, $(dn/dc)_\mu$, was taken to be 0.104 [10]. Data acquisition and molecular weight calculations were performed using the ASTRA software (Wyatt Technologies).

RESULTS AND DISCUSSION

Physical characteristics of the stationary phase

Two different types of macroporous monodisperse particles, designed I and II, were studied. Particle characteristics are given in Table 1. It may be noted that although the

materials have similar particle diameters, the specific pore volumes and specific surface areas are quite different.

The scanning electron micrographs (Figure 1) clearly demonstrate the monodispersity and the spherical shape of the particles. It is visually observed that type II particles contain larger pores than type I, something which is also seen in the pore size distribution curves. The pore size distribution curves in Figure 1 show that the particles have relatively broad pore size distributions whose maxima depend on the particle type. Particle I has a higher proportion of small pores and should therefore have the potential to separate at lower molecular weight than particle II. Particle II contains more pores in the higher regions than particle I, and 88% of the pore volume consists of pores larger than 500 Å, while the corresponding value for particle I is 40%. The higher surface area found for particle I is attributed to the higher amount of smaller pores.

Column efficiency and stability

Upon running cellulose samples continuously for more than 6 months, the number of theoretical plates (N_m) and column back pressure were monitored by regular injections of a polystyrene standard ($M_w = 2.85 \cdot 10^4$). Results for column II+I (serially coupled) are given in Table 2. N_m was initially identical for columns II+I and PLgel Mixed B, and decreased by 4% and 12%, respectively, after 6 months of use.

A backpressure of 100 psi was observed both for columns I and II using LiCl/DMAc at a flowrate of 1.0 ml/min. It increased to 150 psi when serially connected. In comparison, the commercial PLgel Mixed B column had a backpressure of 1060 psi under the same conditions.

Minor increases in the backpressure were occasionally observed in column II+I. This was attributed to accumulation of high molecular weight cellulose. However, the initial backpressure was fully recovered after washing the column with the eluent, or occasionally, with higher LiCl/DMAc concentration of LiCl (up to 6%). Generally, more pronounced increases in the back pressure, and even column plugging, was observed for the PLgel Mixed B column.

Reproducibility of the retention time for column II+I was measured by the injection of a polystyrene standard (M_w=$2.85 \cdot 10^4$). Only very small variations in retention times (typically

1%) and calculated molecular weight were observed after several hundred injections of cellulose interspersed with washing procedures.

The particles seemed to be inert towards chemical degradation, but somewhat more sensitive towards mechanical treatment, as increasing amounts of fine particle fragments were observed after storage and repacking. To remove fragments, repeated sedimentation and decantation of the particle suspension was performed.

Separation ranges (polystyrene standards)

Polystyrene standards were used for assessing and comparing the separation ranges of the different particle types. The selectively coefficient, α, was used as it allows a direct comparison of selectivities of columns with different sizes. α is defined as $(V_e - V_0)/(V_t - V_0)$, where V_e is the peak elution volume, V_0 is the void volume and V_t is the total volume [11].

Figure 2 shows SEC chromatograms obtained with columns I, II and the serial combination of II+I for polystyrene standards with weight average molecular weights (M_w) ranging from 0.004 - $2.0 \cdot 10^6$. It is observed that high molecular weight samples are better resolved with column II as compared to column I, whereas the opposite is the case for low molecular weight samples. As expected, the serial combination (II+I) gave good separation across the entire molecular weight range. It should be noted that polystyrene standards with high molecular weight are relatively polydisperse ($M_w/M_n = 1.3$), giving rise to broader peaks.

The calculated calibration curves (log M versus elution volumes at the peak maximum) are also shown in Figure 2. Here is also included data for the PLgel Mixed B column (chromatograms not shown). The curves are essentially parallel, but are shifted towards higher molecular weight when going from column I to II. The shift is in qualitative agreement with the shift in pore size distribution towards larger pores (Figure 1). The effective separation range for II+I is shifted by a factor of 3.0 in molecular weight (measured at $\alpha = 0.5$) relative to that of the commercial PLgel Mixed B column. Due to the lack of available standards with molecular weights larger than $2.0 \cdot 10^6$, the full separation potential cannot be directly assessed in the high molecular weight region for the MMP particles.

Cellulose samples

Figure 3 shows RI signals and calibration plots (plots of log M versus elution volume as calculated using the ASTRA software) obtained for three separate preparations (unfiltered) of the same cellulose stock solution (SSC, 8% LiCl in DMAc) with 2 parallels of each sample. The sample recovery was estimated to (100 ± 2) % for all 6 injections, indicating no significant adsorption of the cellulose material. The figure shows good reproducibility. The molecular weight was estimated to $(2.12 \pm 0.10) \cdot 10^5$ g/mol.

The dependence of the chromatographic behaviour on the amount of injected cellulose was tested for SSC by injecting concentrations in the range 0.2-1.0 mg/ml. The elution profiles and the corresponding calibration curves (Figure 4) are essentially independent of the amount of sample injected, although the signal-to-noise ratio of the light scattering signal decreases with decreasing sample concentration. The calculated M_w was also independent of the injected amount.

Figure 5 shows the elution curves and the corresponding plots of log M versus elution volume of three high purity cellulose samples of different origin and molecular weight (MCC, SSC, CLC). The difference in elution volume compared to other results presented in this paper is caused by repacking the columns to a different volume. The M_w values of the three samples investigated were calculated to $5.3 \cdot 10^4$, $2.0 \cdot 10^5$ and $8.0 \cdot 10^5$, respectively. The samples were well separated, as demonstrated by the linear decrease in log M with increasing elution volume. The column generally demonstrates good separation according to M in the range from $2.0 \cdot 10^4$ to $2.0 \cdot 10^6$ for cellulose. This is supported by the overlapping calibration plots.

The differential molecular weight distribution is calculated automatically by the software. In order to obtain correct estimates of the MWD, it is necessary that a calibration curve be correctly assigned across the entire distribution. This is usually not the case at the low molecular weight tail because of decreasing signal-to-noise in the light scattering signal. In this case a linear fit was used. The inset in Figure 5 shows the calculated MWD for the three samples. Molecular weight distributions of different wood-based cellulose samples will be further discussed in another work [10].

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TABLES

Table 1: Particle characteristics

Particle type	Particle diameter (μm)	Specific pore volume (ml/g)	Specific surface area (m^2/g)
I	15	1.3	125
II	15	1.94	52

Table 2: Stability of MMP and commercial PLgel Mixed B columns

Particle type	Nm start	Nm after 6 months
II+I	7160 ± 20	6850 ± 20
Mixed B	7180 ± 25	6530 ± 30

FIGURE LEGENDS

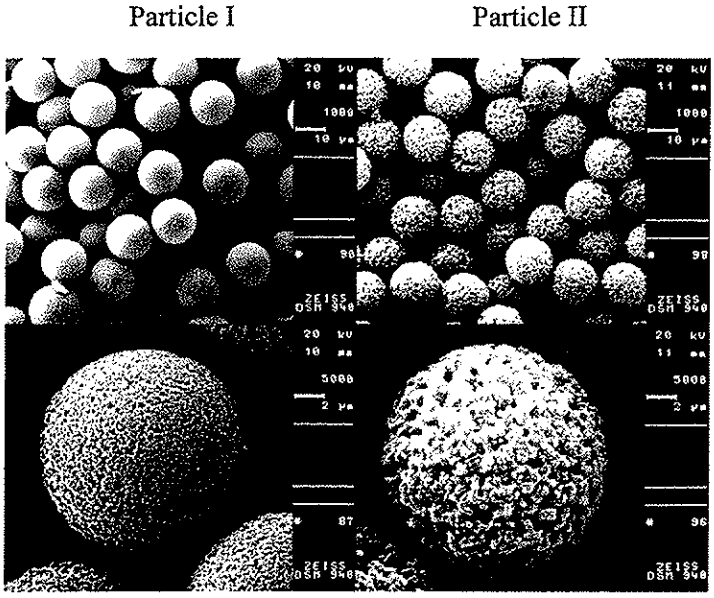
Figure 1. Scanning electron micrographs of MMP cloumn material type I (left) and II (right) and pore size distribution of determined by mercury porosimetry.

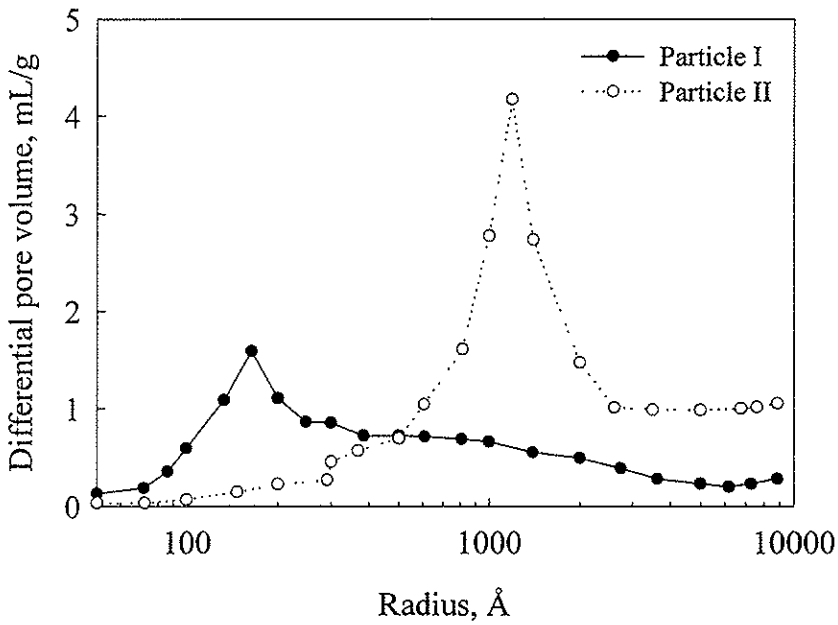
Figure 2. Chromatograms obtained for polystyrene standards (PS1-PS7) using columns I, II and II+I. The molecular weight (M_w) values are given as obtained from the supplier. Calibration plot is given at the bottom.

Figure 3. Chromatograms of three different preparations of the same cellulose sample (SSC). Each preparation was injected twice.

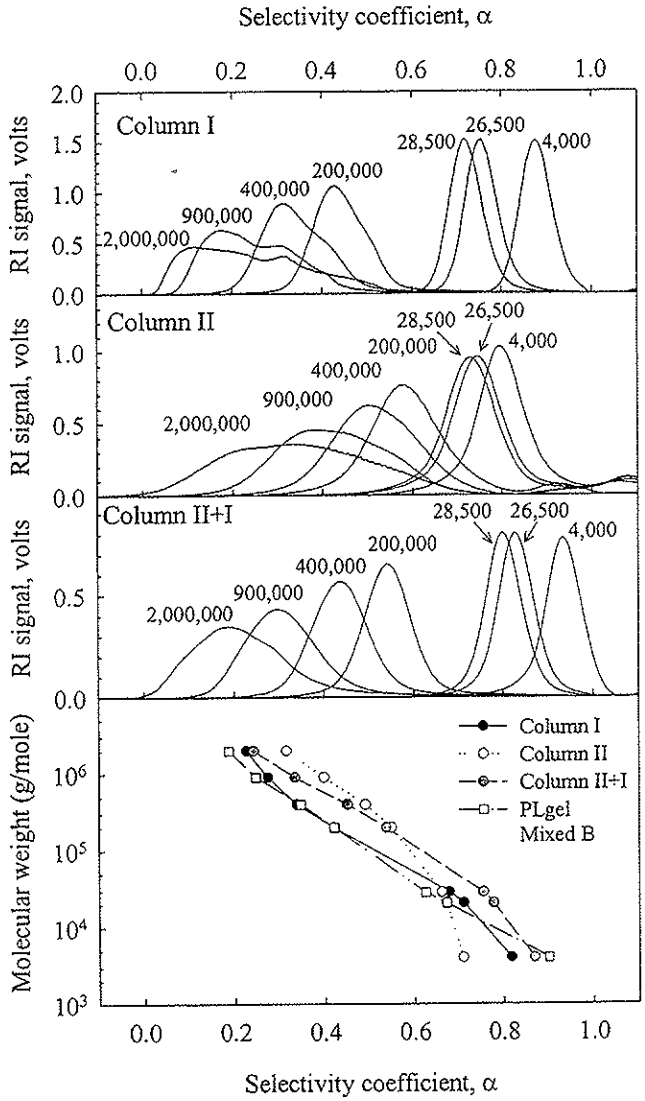
Figure 4. Calibration plots and elution curves for different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) of sample SSC. Sample volume was 200 μl.

Figure 5. Calibration plots and elution curves for the cellulose samples CLC, SSC and MCC. Insert shows the calculated molecular weight distribution (MWD) of the same samples.

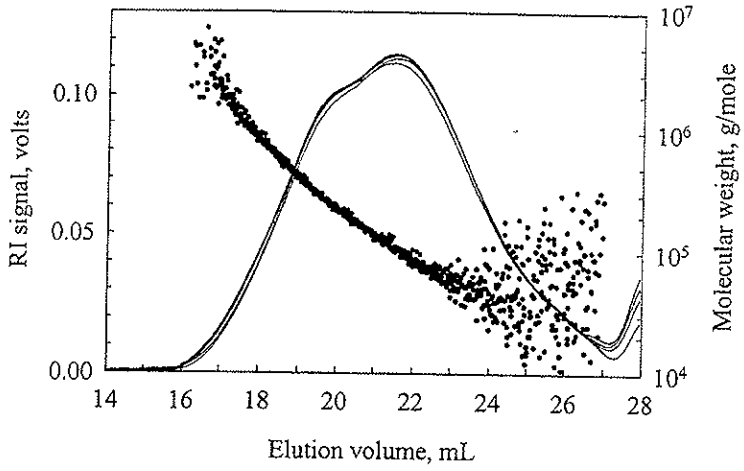




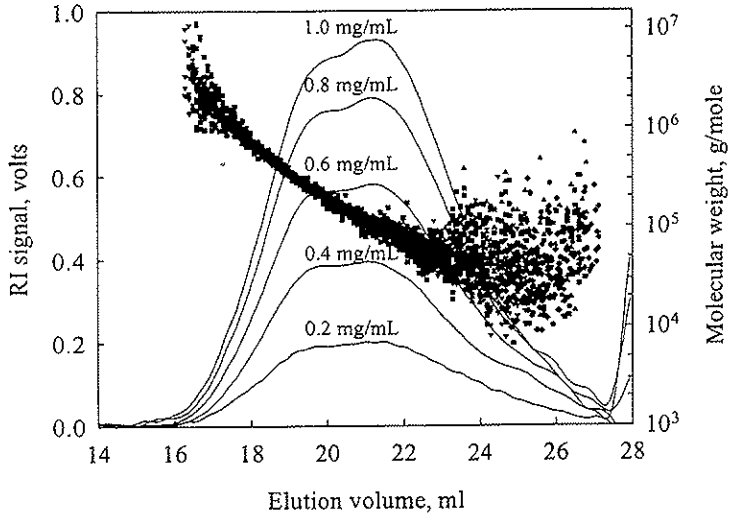
Schult, Moe, Hjerde, Christensen: Size exclusion chromatography of cellulose
Figure 1



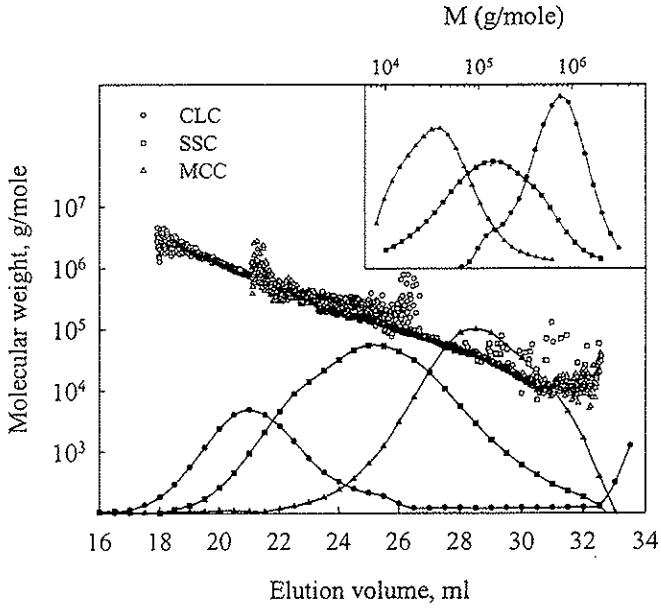
Schult, Moe, Hjerde, Christensen: Size exclusion chromatography of cellulose
Figure 2



Schult, Moe, Hjerde, Christensen: Size exclusion chromatography of cellulose
Figure 3



Schult, Moe, Hjerde, Christensen: Size exclusion chromatography of cellulose
Figure 4



Schult, Moe, Hjerde, Christensen: Size exclusion chromatography of cellulose
Figure 5

PAPER II

Characterization of cellulose by SEC-MALLS

Tove Schult[a)], Torgeir Hjerde[b)], Odd Inge Optun[c)], Peder J. Kleppe[a)] and Størker T. Moe[a),*)]

[a)] Department of Chemical Engineering, Norwegian University of Science and Technology (NTNU), N-7491 Trondheim, Norway

[b)] Borregaard ChemCell, P.O. Box 162, 1701 Sarpsborg, Norway

[c)] SINTEF Unimed MR Center, 7465 Trondheim, Norway

[*)] Author for correspondence

Abstract

Norway spruce (*Picea abies*) cellulose samples dissolved in in lithium chloride/*N,N*-dimethylacetamide (LiCl/DMAc) covering a wide range of average molecular weights were analyzed by size exclusion chromatography (SEC) and multi-angle laser light detection (MALLS). The molecular weight distribution of the samples was compared to the molecular weight distribution of cotton linters cellulose samples. To obtain complete dissolution of high-molecular weight wood cellulose, the procedure for dissolving cellulose in LiCl/DMAc was modified, and the modified procedure is reported. SEC separation was performed using macroporous monodisperse polymer particles as column matrix. The refractive index increment $(dn/dc)_\mu$ for cellulose in 0.5% LiCl/DMAc was measured to 0.104. The radius of gyration, R_G, of cellulose in 0.5% LiCl/DMAc depended on the molecular weight, M according to the relation $R_G \propto M^{0.55}$. Cellulose prepared from sprucewood by the sulfite cooking process had a broad molecular weight distribution compared to cotton linters cellulose.

Keywords

Cellulose, wood, cotton, SEC, chromatography, GPC, light scattering, MALLS, SEC-MALLS, molecular weight, molecular weight distribution, dimethylacetamide, DMAc, LiCl/DMAc, DMAc/LiCl, conformation, radius of gyration, R_G, dn/dc, refractive index increment

Introduction

Cellulose, probably the most abundant biopolymer in the world, is present in all higher plants and exists in its purest naturally occurring form in cotton fiber. Its characterization is of particular interest to the textile, pulp and paper, and cellulose derivative industries.

Cellulose consists of (1-4)-β-D-glucopyranose units linked linearly to form long chains. Unlike other polysaccharides (e.g. hemicelluloses, high molecular weight dextrans and pullulans), it shows no variability in structure over its entire molecular weight (MW) range. Observed differences between cellulose samples originate mainly from differences in molecular weight distributions (MWDs) and supramolecular structure. Due to the various processing conditions required to obtain high-purity celluloses from different raw materials, differences in molecular weight distribution will be expected for cellulose samples isolated from various raw materials.

The crystalline structure of cellulose is stabilized by inter- and intramolecular hydrogen bonds. The dense crystalline structure and the high number of intramolecular hydrogen bonds makes

cellulose virtually insoluble in most common solvents (1). Due to the low solubility of cellulose, MWD analysis of cellulose by SEC is not a trivial task. *N,N*-dimethylacetamide containing lithium chloride (LiCl/DMAc) seems to be the most promising solvent for cellulose (2-6) and is considered to be a non-degrading solvent (7,8), although a slight decrease in viscosity has been reported after 30 days in solution (6). Originally, the solvent was used to dissolve chitin (9) and cellulose derivatives (10) but has now become a favored solvent in the analysis of several polysaccharides (11). However, in practice, cellulose has a limited solubility also in this solvent, especially at high molecular weights, something which promotes the existence of aggregates (3,12).

Problems associated with SEC analysis of cellulose like incomplete dissolution, formation of aggregates and precipitation of cellulose on the column matrix have been reported (3,13-14). Some of the problems in SEC may be eliminated by choosing a suitable column material (15) while better solubility requires improvements in the procedure and conditions for dissolving cellulose in LiCL/DMAc. In this work, macroporous monodisperse polymer particles (MMP) were used as a column material for SEC separation of cellulose. This column material has has been applied successfully for SEC analysis of polysaccharides in an aqueous system (16), and lately also for cellulose in LiCl/DMAc (17).

LiCl/DMAc as a solvent for cellulose

LiCl has the ability to form ion pairs, which are characterized by being electrostatically linked in polar aprotic solvents. Since anions are poorly solvated and cations are strongly solvated by solvent molecules, the solvation of the small lithium cation by DMAc is interpreted as a complex formation. This is mediated through interaction between the Li^+ ion and the carbonyl oxygen of DMAc. However, different views exist on the detailed structure of these LiCl-DMAc complexes (18). It seems likely that the stability of these complexes is the crucial factor for dissolution of cellulose. Thus, if the complexes are either too strong or too weak, LiCl/DMAc loses its potential to act as a solvent for cellulose. The complexes are otherwise assumed to interact strongly with the hydroxyl groups of cellulose. Each hydroxyl group of the anhydroglucose unit may be approached by a single LiCl-DMAc complex (19). The result is that hydrogen bonds are broken and that the carbohydrate's hydroxyl groups may be solvated by forming complexes with the LiCl-DMAc complex.

A number of models for the dissolution of cellulose in LiCl/DMAc have been proposed (18), and the majority emphasize the key role played by the LiCl ion pairs in the formation of complexes. It has been proposed that the chloride ions of solvated ion pairs, $(Li \cdot xDMAc)^+Cl^-$, are free to establish hydrogen bonds with hydroxyl protons of cellulose, leading ultimately to cellulose dissolution (6). Another mechanism has been proposed where the interaction of the chloride anion with the hydroxyl protons would result in competitive hydrogen bond to the intermolecular hydrogen bond structure in cellulose. A further association of Cl^- along the cellulose chain would produce an anionically charged polymer with the macrocation $(Li\text{-}DMAc)^+$ as the counterion and subsequent dissolution of cellulose (19). Although much is understood about the interaction between LiCl/DMAc and carbohydrate molecules, a great deal remains to be known in order for a comprehensive mechanism of dissolution to be discerned.

Scope of the work

The purpose of this work has been to obtain information on molecular conformation and molecular weight distributions of wood-based high molecular weight celluloses by SEC-MALLS. Differences in the MWDs of the pulps may then be explained by considering the

effect of the chemical processes during manufacture of high-purity cellulose pulps from spruce wood by sulfite pulping and subsequent bleaching. A comparison of the wood-based celluloses with cotton linters cellulose samples of comparable SCAN viscosity numbers is illustrative when considering the effect of processing conditions and fiber morphology type on the MWD of cellulose samples.

Experimental

Materials

Cellulose samples were eight industrially prepared wood-based cellulose samples produced by the acid sulfite process, obtained from Borregaard Industries, Ltd. (designated D1-D8) and four commercial grades of cotton linters obtained from Peter Temming AG (designated C1-C4). The intrinsic viscosity of the cellulose samples was determined according to the SCAN standard (SCAN CM-15:88 (20)). This method is based upon a single point measurement of viscosity dissolved in 0.5% CED (copperethylenediamine) solution and the use of correlation tables to obtain the intrinsic viscosity of the sample. Intrinsic viscosities of cellulose samples are given in Table 1.

Materials used for dissolving cellulose were DMAc (p.a. grade, Burdick & Jackson) and methanol (p.a. grade, Sigma-Aldrich), both dried with molecular sieves (Baker Type 4A, activated by heating to 450°C for 3 days.), acetone (p.a. grade, Acros), EDTA (ethylene diamine tetraacetic acid, 99%, Fluka), DTPA (diethylene triamine pentaacetic acid, 99%, Fluka), citric acid (p.a. grade, Merck), LiCl (analytical grade, Baker, ovendried at 150°C) and deionized water.

Sample preparation

The pulp samples were disintegrated in deionized water using a laboratory disintegrator. The samples were then swollen in 0.1M LiCl under vacuum overnight and subsequently washed with 2% aqueous EDTA, 2% aqueous DTPA and finally with 5% aqueous citric acid. The cellulose was further washed with 0.1M LiCl followed by an acetone extraction. The sample was then solvent exchanged with methanol and then DMAc under absolute dry conditions by Soxhlet extraction. Simultaneously, an 8% solution (w/v) of LiCl in DMAc was made by continuous stirring and heating to approximately 80 °C. The pulp was directly added to the 8% LiCL/DMAc solution and continuously stirred for 4 hours at room temperature. The solution was then allowed to stand for up to 5 days at 4°C to achieve complete dissolution of the cellulose. Before injection, the solution was diluted to 0.5% DMAc/LiCl and filtered through a solvent resistant, disposable teflon filter (Millipore Millex SR 0.5mm; for the highest molecular weight cellulose samples Millipore Millex CN 3.0 mm were used).

Size exclusion chromatography

SEC was performed at 40°C using a Perkin Elmer (Series 2000) HPLC pump with autosampler, operating at a flow rate of 1.0 ml/min. Injected samples contained 50-200 mg of dissolved cellulose. The elution profile was monitored by a refractive index detector (Shimadzu RID-10A) and a multi-angle laser light scattering detector (MALLS, Wyatt Dawn DSP, 633 nm) equipped with an in-line filter holder (Millipore) with 0.2mm PTFE-filter (Fluoropore-FG, Millipore). Data acquisition and molecular weight calculations were performed using the ASTRA software (Wyatt Technologies).

Refractive index increment measurements

The refractive index increment, $(dn/dc)_\mu$, of spruce and cotton linters cellulose in 0.5% LiCl/DMAc was determined at 40°C using a Shimadzu differential refractometer (Shimadzu RID-10A) which was connected to a thermostatic water bath. The instrument was calibrated with KCl and polystyrene solutions with known refractive indices. Samples of wood cellulose with exact known concentrations were then injected and the refractive index increment was calculated automatically using the ASTRA software. The measured values of $(dn/dc)_\mu$ were then used in the SEC/MALLS/RI experiments.

NMR experiments

NMR diffusion spectra were recorded for solutions of 1.925% cellulose in LiCl/DMAc. Reference sample was the solvent (LiCl/DMAc) without cellulose added. The NMR spectrometer was a Bruker AMX500 spectrometer operating at 500 MHz equipped with standard pulsed field z-gradient (PFG) acessory. The diffusion spectra were generated using a stimulated echo experiment with LED (longitudinal eddy delay) (21). The diffusion spectra were obtained with these parameters: 168 scans, 32 increments, 8K time domain, maximum gradient strength of 50 G/cm^2, gradient on time: 5 ms, diffusion time 60 ms and eddy current delay (Te) = 50 ms.

Results and Discussion

Dissolving cellulose in LiCl/DMAc

Several methods for dissolving cellulose in LiCl/DMAc have been proposed (6,19,22). Variations over these procedures were tested, but a direct application was unsuccessful for a total dissolution, especially of high molecular weight samples. A new modified method was required for the analysis of the acid sulfite pulps, and the procedure was optimized through the experimental variables listed in Table 2. Only a few combinations of the variables and conditions produced soluble samples.

The formation of the Li^+-(DMAc)-Cellulose-Cl^- complex may be hindered by metal ions. Therefore, deionized water was used in the disintegration, swelling and washing steps. Several activation (swelling) agents were tested (pure water, LiCl and NaOH). LiCl was the most promising and gave up to 10% more cellulose dissolved in 8% LiCl/DMAc than what water gave.

Metal ions are naturally associated to native cotton and wood by ionic bonding to carbohydrates. Remaining ions after pulping were removed by complexbinder treatment (EDTA, DTPA and citric acid). Several combinations of chelating agents, different concentrations of agents and washing times were investigated. The results showed that the concentration of chelating agent was of minor importance. Washing times for each chelating agent showed only minor effect after 30 min. The order of agents used was important, especially for high molecular cellulose samples. The optimal order was found to be EDTA, followed by DTPA and finally by citric acid. The introduction of a separate LiCl step was moved to after the treatment with chelating agent to make sure that any ions associated with the pulp were Li^+ ions. The acetone extraction was performed to remove possible extractives left in the pulp but also as a first stage in the solvent exchange. The solvent was changed through methanol to DMAc by soxhlet extraction.

The solubility of cellulose is known to increase as the LiCl concentration increases (6). Sample concentrations between 0.5 and 1.5 mg/ml (cellulose/DMAc) were tested and compared at different concentrations of LiCl (6, 8, 9 and 12%) in the solvent system. At a LiCl concentration of 6% weight/volume, insoluble particles appeared in the solution. With a LiCl concentration of 8%, a complete dissolution of cellulose occurred. For the highest molecular weight samples, a sample concentration of 0.5 mg/ml was preferable.

After preparation of 8% LiCl/DMAc and addition of cellulose, the suspension was stirred for 4 hours before the sample was allowed to stand for 1-5 days at 4 °C (23) to obtain complete dissolution. According to the literature, longer activation and stirring times are necessary for dissolving cellulose samples with a high degree of crystallinity (α-cellulose content), MW and lignin content (4). Fig. 1 shows a flow diagram of the final dissolution procedure of acid sulfite pulps and cotton linter cellulose used in the SEC analysis later in this work.

SEC analysis

Adsorption of cellulose to the stationary phase has been a major problem in SEC separation of cellulose. This problem may be reduced by choosing more suitable column materials. The application of macroporous monodisperse particles in the separation of cellulose has been successful (17) and has therefore been used in this work.

Determination of the refractive index increment, $(dn/dc)_\mu$, of cellulose

The determination of $(dn/dc)_\mu$ for cellulose in 0.5% LiCl/DMAc was performed indirectly using the ASTRA software (Wyatt Corp.). Solutions of cellulose sample D1, which is easily soluble in 8% LiCl/DMAc, were prepared, and the diluted samples were injected onto the columns (80 mg) without prior filtration. The $(dn/dc)_\mu$-values were calculated in the software by assuming 100% recovery of the injected material and a known calibration constant for the RI-detector (RI_c):

$$\left(\frac{dn}{dc}\right)_\mu = \frac{\sum_i V_i \cdot RI_c}{\sum_i c_i} \qquad \text{Eq. 1}$$

where V is the RI detector output voltage and c is the polymer concentration. The value based on the average of 6 injections was 0.104 ± 0.002 ml/g.

Measuring the chain flexibility of cellulose in 0.5% LiCl/DMAc

According to classical polymer theory, a flexible polymer (random coil) under Flory theta (θ) conditions has a radius of gyration which is proportional to the square root of the molecular weight of the polymer (Eq. 2).

$$R_G \propto M^\alpha, \alpha = 0.5 \qquad \text{Eq. 2}$$

For random coils in good solvents, the exponent α in equation 1 varies between 0.5 and 0.6, while higher values of α ($0.6<\alpha<1.0$) are considered an indication of a stiff coil or rod-like conformation. In SEC-MALLS experiments, it is possible to obtain the radius of gyration for each SEC elution slice (Fig. 2a and 2b), and the relationship between molecular weight and radius of gyration can thus be obtained. A linear regression of the data provides the scaling factor (α) which was calculated to 0.55 for cellulose in 0.5% LiCl/DMAc. The relation between molecular weight and radius of gyration can thus be expressed by Eq. 3:

$$R_G \propto M^{0.55 \pm 0.01} \qquad \text{Eq. 3}$$

Although no data have been found in the literature on the direct relation between R_G and M for cellulose in LiCl/DMAc, published values of the MHS exponent for cellulose in LiCl/DMAc may be used for comparison. Using the equivalent sphere model for polymer solution viscosity (Eq. 4) (24), the value of α measured in this work of 0.55 corresponds to a value for the MHS exponent a of 0.65.

$$[\eta] \propto \frac{R_G^3}{M} \qquad \text{Eq. 4}$$

Considering that the MHS exponent a of cellulose in 8% LiCl/DMAc previously has been determined to 1.19 (6), a value of α of 0.55 is surprisingly low. It was, however, shown in the same work that the intrinsic viscosity of a cellulose sample in LiCl/DMAc is strongly dependent on the LiCl concentration and decreases with decreasing LiCl concentration. This indicates that the cellulose molecule adopts a less extended conformation at low LiCl concentrations. The good correspondence between the data for both wood cellulose samples (Fig. 2a) and for cotton linters cellulose samples (Fig. 2b) supports the results reported in this paper.

Traditionally, cellulose is considered to be a molecule with a large degree of inherent chain stiffness, as demonstrated by published values for the MHS exponent of 1.19 in 8% LiCl/DMAc and 0.905 in CED solution [25]. However, one of the reasons for the high chain stiffness of cellulose is the presence of intramolecular hydrogen bonds. A weakening of these hydrogens bonds could therefore lead to an increased freedom of rotation around the glycosidic bond and consequently to a lowering of the inherent chain stiffness. Considering that one hypothesis for cellulose dissolution in LiCl/DMAc involves the interaction between the $(Li \cdot xDMAc)^+Cl^-$ complex and hydroxyl groups on the glycosidic ring, it may be likely that intramolecular hydrogen bonds may be weakened through interaction with the $(Li \cdot xDMAc)^+Cl^-$ complex.

NMR diffusion experiments show that the diffusion of DMAc in the cellulose/LiCl/DMAc system is different from DMAc in the pure LiCl/DMAc system, as shown in Fig. 3 where the attenuation of DMAc in LiCl/DMAc is higher than for DMAc in the cellulose/LiCl/DMAc system. This difference in diffusivity is an indication that a fraction of the DMAc in the cellulose-LiCl/DMAc solution is closely associated with the cellulose, probably through interactions between DMAc and cellulose which may weaken hydrogen bonds in the cellulose molecule.

Other factors like segment-segment interactions (intermolecular association) may also significantly decrease the hydrodynamic volume of the cellulose molecule and give an α value closer to that which would be expected for a more flexible chain. One cannot therefore safely conclude that cellulose adopts a random coil configuration in 0.5% LiCl/DMAc. It seems, however, that the cellulose molecule has a significantly less extended chain configuration under these conditions than has been determined under other conditions.

Molecular weight distributions of cellulose samples

The various methods for preparing high-purity cellulose samples for commercial use should be expected to give cellulose samples of varying molecular weight distributions. So far, no data have been found in the literature regarding molecular weight distributions of high-molecular weight wood-based celluloses. This may be due to difficulties in obtaining a true solution of

such cellulose samples, as mentioned previously. Fig. 4 shows the elution curves and corresponding plots of log M versus elution volume of three selected wood-based cellulose samples (D1, D5 and D8) of different viscosity-average molecular weight ($\overline{M}_v$). The samples were well separated, as demonstrated by the linear decrease in log M with increasing elution volume. The columns used in this study give good separation according to M in the range $2.0 \cdot 10^4 - 1.0 \cdot 10^7$ g/mole for cellulose (17). This is supported by the overlapping calibration plots obtained in this work (Fig. 4 and Fig. 5). The sample recoveries were calculated to 100% for low molecular weight cellulose and 92 ± 2% for high molecular weight cellulose. MALLS data shows little light spreading which indicates that the cellulose has been well diluted. The calculated $\overline{M}_w$ value and polydispersity index (PI, $\overline{M}_w/\overline{M}_n$) of every acid sulfite pulp sample analyzed is shown in Table 3.

The large difference in molecular weight distribution between the wood-based (Fig. 4) and cotton linters (Fig. 5) cellulose samples illustrated in Table 4 can be explained by considering differences in both fiber morphology and process conditions during manufacture for the two types of cellulose. Cotton linters consist predominatly of highly crystalline cellulose, and only mild alkaline digestion and bleaching is required to give a cellulose product of acceptable purity. Softwood tracheids ("fibers"), on the other hand, consists of approximately 40% cellulose, while the remainder of the fiber components are hemicelluloses and lignin. A strong chemical treatment is thus necessary to give a pulp with a high content of cellulose. In the acid sulfite process, which has been used for the manufacture of the samples investigated in this work, the wood raw material is treated ("cooked") with a strongly acidic cooking acid consisting of aqueous SO_2 and Ca-bisulfite at high temperatures (120-150 °C). Under acidic conditions, one will have a non-random hydrolytic attack on the amorphous regions of the cellulose molecules, and a broad molecular weight distribution will be expected. In addition, the fiber wall of the tracheids has a comparatively larger proportion of amorphous hemicelluloses and lignin in the outer parts of the cell wall. Thus, acidic hydrolysis will preferably take place in the outer parts of the fiber wall and a further broadening of the molecular weight distribution should be expected (26,27).

Fig. 6 shows the molecular weight distributions of seven of the wood cellulose samples. One sample (D5) has been omitted from this figure due to different process conditions during manufacture. The other samples have been manufactured using the same chemical composition of the cooking liquor and the same sequence of bleaching chemicals, albeit with slightly different process condtions in order to produce pulps with different average molecular weight. These samples should thus be directly comparable. One can notice that together with the shift in average molecular weight to lower values, a slight trend towards a narrower MWD can be observed.

Chemical treatment of the pulp after cooking by bleaching chemicals should not affect the MWD of the cellulose to a large degree compared to the cooking conditions, due to the higher selectivity of the bleaching chemicals. However, certain types of treatment, like alkaline extraction, can be used to solubilize a part of the low-molecular weight polysaccharides and give a narrower MWD. This can be seen for two of the sulfite pulp samples (sample D6 and D3), where process conditions have been optimized to give a pulp with a high fraction of α-cellulose, i.e. a high proportion of crystalline, high-molecular weight cellulose. The bimodal distribution of the high molecular weight pulps like samples D7 and D8 would be expected for a non-random degradation where a certain fraction of the cellulose molecules is subject to strong degradation, while another fraction is in some way protected from acidic hydrolysis. Of

course, the low-molecular weight peak may also be attributed to hemicelluloses, which are reported to have significantly lower molecular weights than what cellulose has (28).

Conclusions

A modified procedure for dissolving cellulose in LiCl/DMAc has been developed. The main feature of the modified procedure was a washing treatment using chelating agents to remove possible metal iones left in the pulp. The order of the chelating agents EDTA, DTPA and citric acid was important.

By assuming 100% recovery of cellulose sample, the $(dn/dc)_\mu$ for cellulose in 0.5% LiCl/DMAc was measured to 0.104 ± 0.002 ml/g. In the same solvent, the radius of gyration of cellulose molecules depended on the molecular weight according to the equation $R_G \propto M^{0.55}$.

The molecular weight distributions of wood-based cellulose samples prepared by the acid sulfite process are broad and, for the highest molecular weight samples, more or less bimodal, something which can be explained by the assumed effect of process chemicals on the cellulose in the wood raw material

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Figure legends

Figure 1: Flow diagram for solution procedure for high-molecular weight cellulose

Figure 2a: Radius of gyration for spruce sulfite pulp fractions, obtained by SEC-MALLS

Figure 2b: Radius of gyration for cotton linters cellulose fractions, obtained by SEC-MALLS

Figure 3: NMR diffusion measurements of LiCl/DMAc with and without cellulose in solution. ○: DMAc in LiCl/DMAc; ●: DMAc in cellulose/LiCl/DMAc (1.925% cellulose); ■: cellulose in cellulose/LiCl/DMAc (1.925% cellulose)

Figure 4: SEC chromatograms and MALLS calibration curves for three selected spruce sulfite pulp samples. Intrinsic viscosities are: D-8: 1550 mL/g; D-5: 1200 mL/g; D-1: 380 mL/g

Figure 5: SEC chromatograms and MALLS calibration curves for four different cotton linters cellulose samples. Intrinsic viscosities are: C-4: 1900 mL/g; C-3: 1550 mL/g; C-2: 1200 mL/g; C-1: 380 mL/g

Figure 6: Molecular weight distributions of seven spruce sulfite samples produced by acid sulfite cooking to different times

Table legends

Table 1: Intrinsic viscosities of cellulose samples

Table 2: Operation variables and treatment for dissolving cellulose samples

Table 3: Weight-average molecular weight, $\overline{M}_w$ and polydispersity index, $\overline{M}_w/\overline{M}_n$, of spruce sulfite pulp samples, calculated from SEC-MALLS data

Table 4: Calculated $\overline{M}_w$, $\overline{M}_n$ and $\overline{M}_w/\overline{M}_n$ for spruce sulfite pulp and cotton linter cellulose with comparable intrinsic viscosities

Tables

Table 1

Sample	Intrinsic viscosity, ml/g
C-1	380
C-2	1200
C-3	1550
C-4	1900
D-1	380
D-2	520
D-3	570
D-4	750
D-5	1200
D-6	1350
D-7	>1450
D-8	>1550

Table 2

Variable	Agent/conditions	Result
Activation agent	H_2O	+
	LiCl	++
	NaOH	0
Activation time (min.)	0	0
	30	+
	60	+
	90	+
Washing treatment	EDTA, DTPA, citric acid, LiCl	The order of washing agents affected the result[a]
Extractive removal	Acetone	+
Sample concentration (mg/ml)	0.5	+
	1.0	+
	1.5	0
LiCl concentration in DMAc (% w/w)	6	+
	8	++
	9	++
	12	+
Stirring time (h)	4	+
	12	+
	24	+
Temperature	room temperature	+
	4 °C	++
Time (days)	1-5	Dependent on molecular weight

a. See text

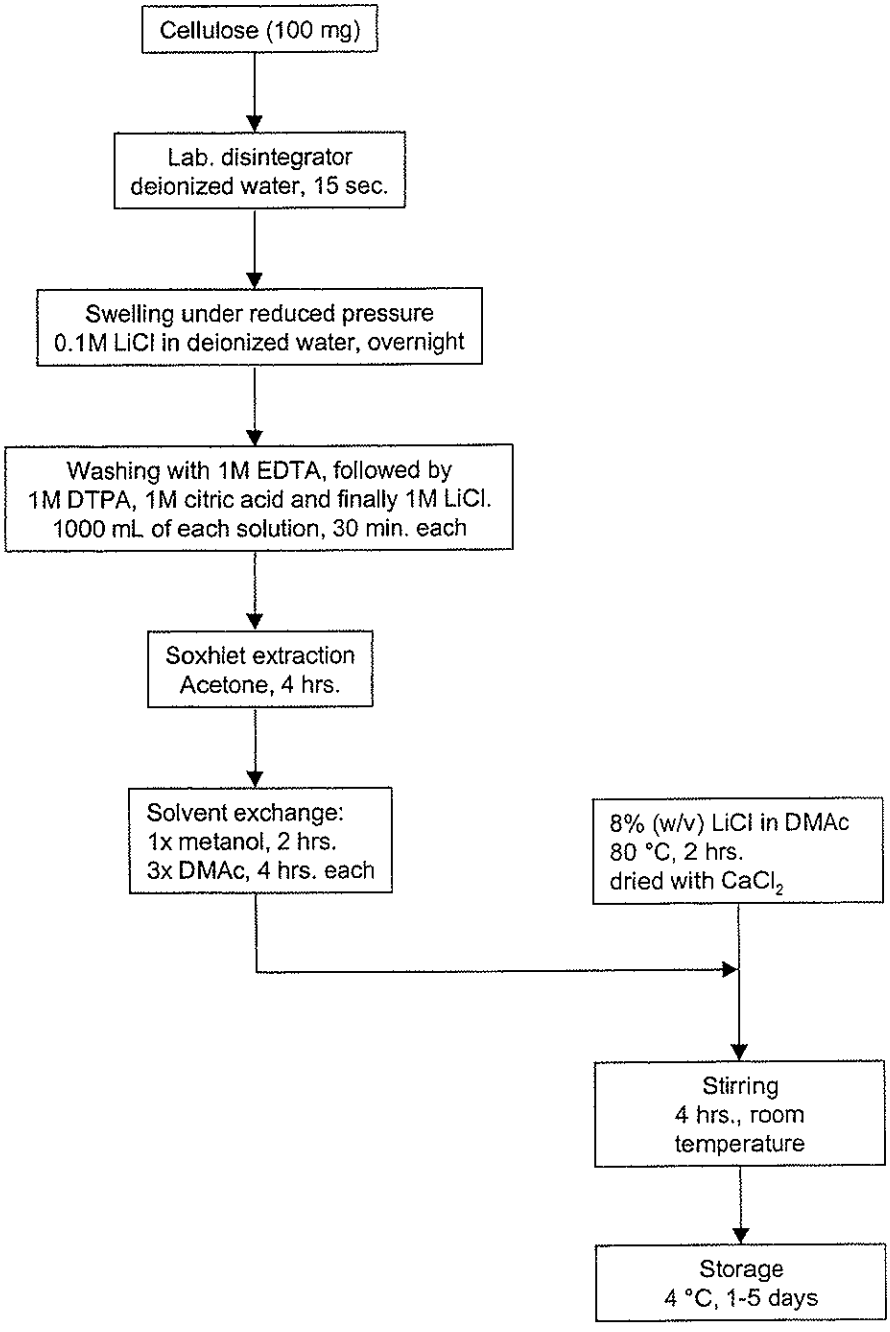
Table 3

Sample	$\overline{M}_w$ ($\cdot 10^3$) g/mole	$\overline{M}_w/\overline{M}_n$
D1	240	3.0
D2	400	5.4
D3	450	2.4
D4	690	5.5
D5	1030	5.4
D6	1300	3.0
D7	1450	5.7
D8	1550	4.9

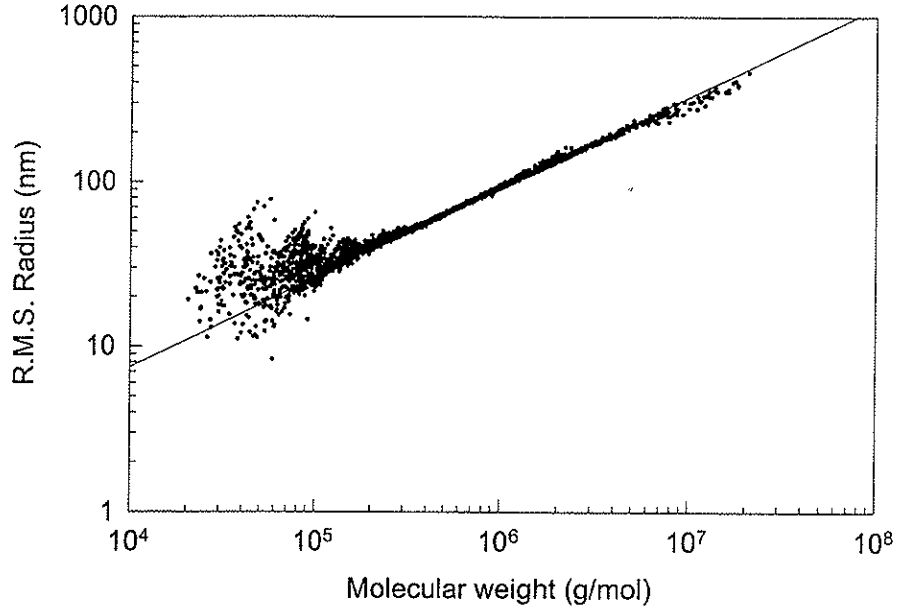
Table 4

Intrinsic viscosity, $[\eta]_{SCAN}$ (ml/g)	Wood cellulose			Cotton linters cellulose		
	$\overline{M}_w \cdot 10^{-3}$ (g/mole)	$\overline{M}_n \cdot 10^{-3}$ (g/mole)	$\overline{M}_w/\overline{M}_n$	$\overline{M}_w \cdot 10^{-3}$ (g/mole)	$\overline{M}_n \cdot 10^{-3}$ (g/mole)	$\overline{M}_w/\overline{M}_n$
380	240	80	3.0	180	100	1.8
1200	1030	191	5.4	890	330	2.7
1550	1550	316	4.9	1370	685	2.0
1900	-	-	-	1750	1167	1.5

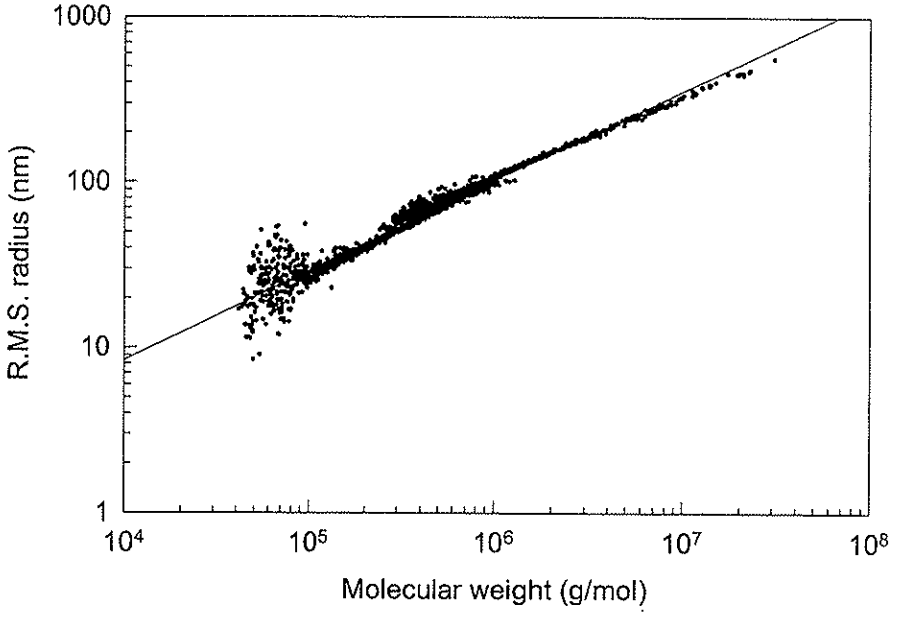
Figures



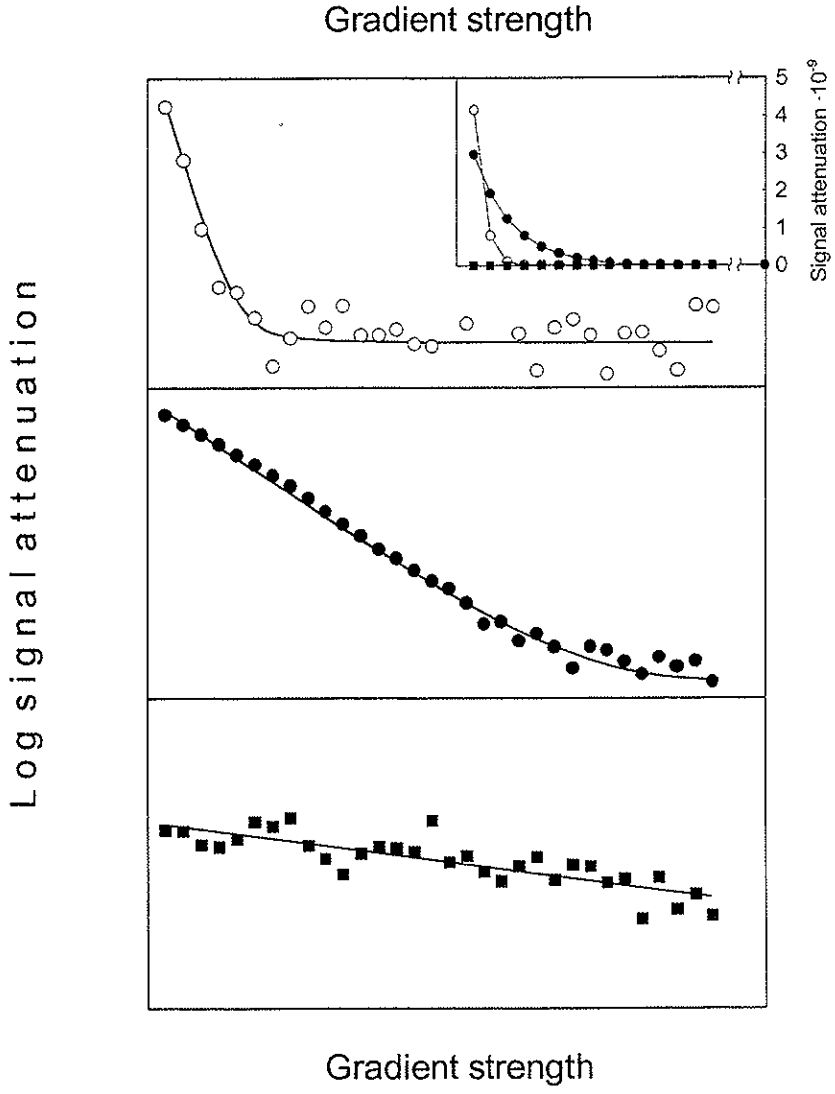
Schult et al: Characterization of cellulose by SEC-MALLS. Figure 1



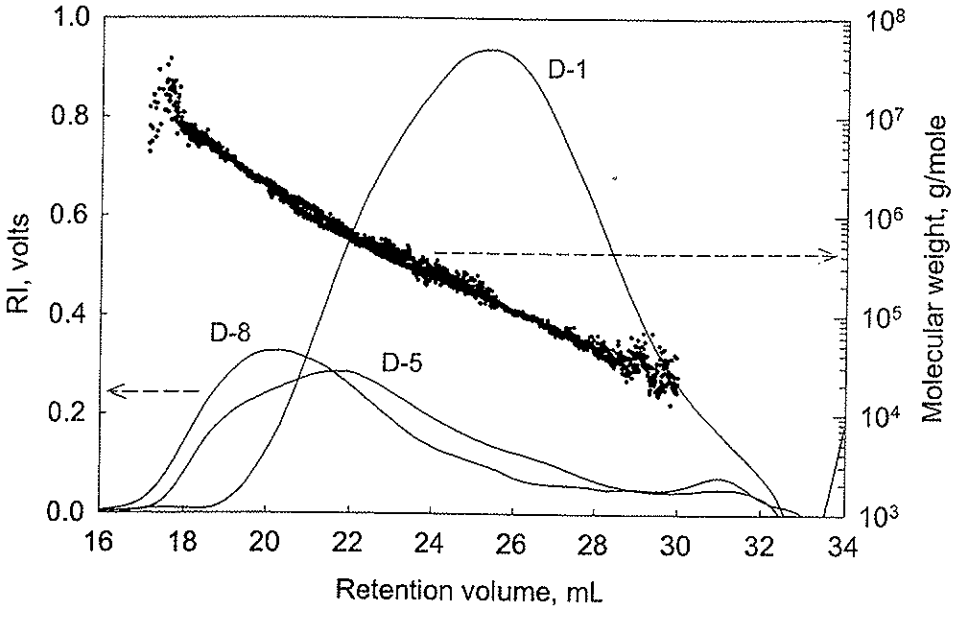
Schult et al: Characterization of cellulose by SEC-MALLS. Figure 2a



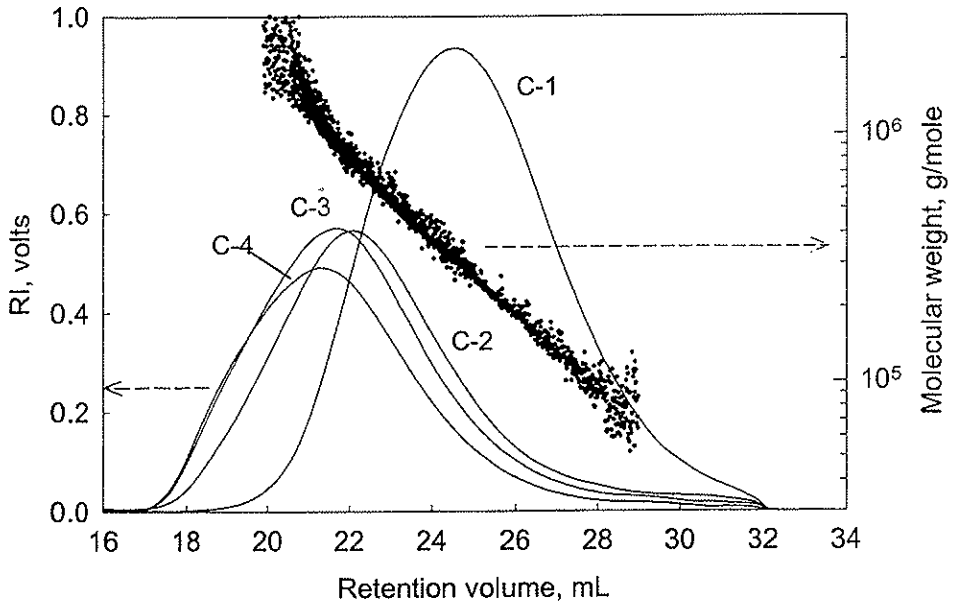
Schult et al: Characterization of cellulose by SEC-MALLS. Figure 2b



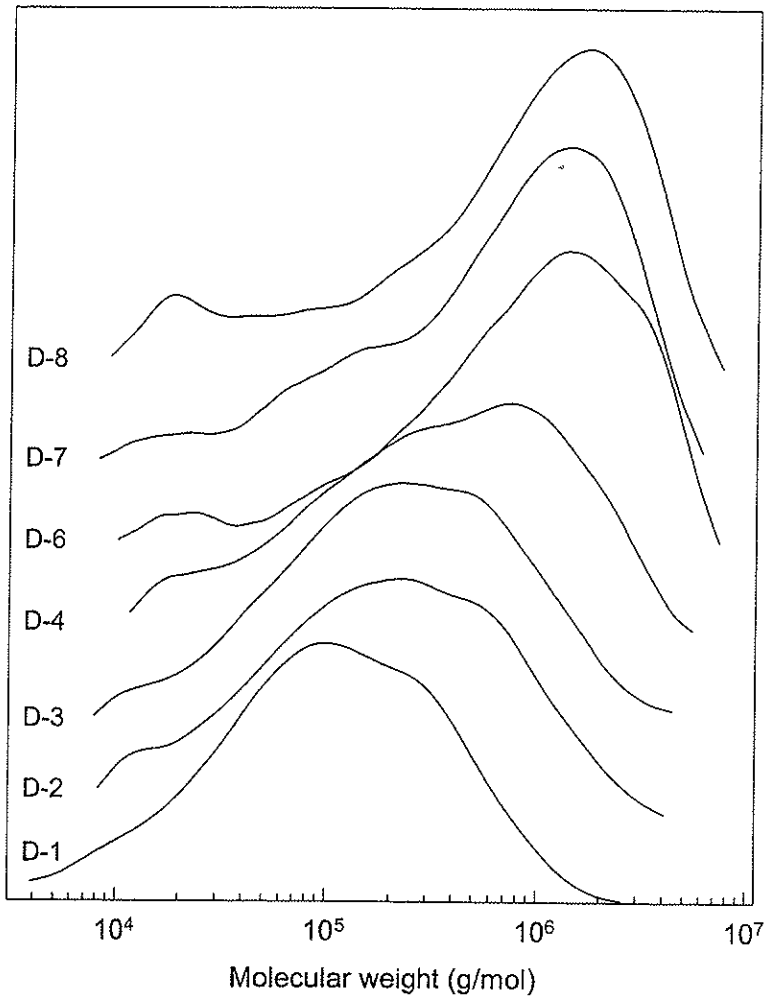
Schult et al: Characterization of cellulose by SEC-MALLS. Figure 3



Schult et al: Characterization of cellulose by SEC-MALLS. Figure 4



Schult et al: Characterization of cellulose by SEC-MALLS. Figure 5



Schult et al: Characterization of cellulose by SEC-MALLS. Figure 6