Novel Cationic Esters derived from Cellulose and Dextran:

Synthesis, Structure Analysis and Properties

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“The real voyage of the discovery consists not in seeking new landscapes,

but in having new eyes.”

Marcel Proust (1871 – 1922)

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Abbreviations

AGU	anhydroglucose unit
AMIMCl	1-allyl-3-methylimidazolium chloride
aq	aqueous
BMIMCl	1-butyl-3-methylimidazolium chloride
CDI	*N,N'*-carbonyldiimidazole
CMC	carboxymethyl cellulose
cont	continuation
CPTAC	(3-carboxypropyl)trimethylammonium chloride
^{1}H/^{1}H COSY	homonuclear correlation spectroscopy
Cuen	cupriethylenediamine
d	day
DEPT	distortionless enhancement of polarisation transfer spectroscopy
DMAc	*N,N'*-dimethylacetamide
DMF	*N,N*'-dimethylformamide
DMSO	dimethylsulfoxide
DMSO-d_6	deuterated dimethylsulfoxide
DP	degree of polymerization
DP_{cuen}	degree of polymerization determined in cupriethylenediamine
DP_n	number average degree of polymerization
DP_w	weight average degree of polymerization
DS	degree of substitution
EA	elemental analysis
EMIMCl	1-ethyl-3-methylimidazolium chloride
Eq	equation

Fig	figure
FTIR	fourier-transform infrared spectroscopy
g	gram
h	hour
HEC	hydroxyethyl cellulose
HSQC	heteronuclear single-quantum correlation spectroscopy
IL	ionic liquid
K_a	acidity equilibrium constant
L	liter
LD_{50}	median lethal dose of 50% of species
M	molar (mol/L)
m	medium
$\overline{M}$	molar mass
$\overline{M}_n$	number average molecular mass
$\overline{M}_w$	weight average molecular mass
MABA	4-[methylammonium]butyric acid chloride
MIC	minimal inhibitory concentration
min	minute
mp	melting point
MS	molar degree of substitution
MTG	methyl 6-O-tosyl-α-D-glucopyranoside
nd	not determined or not detectable
NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
Nr	number

PDADMAC	polydiallyldimethylammonium chloride
PDI	polydispersity index
pK_a	negative decadic logarithm of K_a
PVS	polyvinylsulfonic acid
Py	pyridine
QCM-D	quartz crystal microbalance with dissipation monitoring
RT	room temperature
®	registered trademark
s	second
SCA	static contact angle
SEC	size-exclusion chromatography
TBAF	tetrabutylammonium fluoride trihydrate
TLC	thin layer chromatography
TOC/TN	total organic carbon and nitrogen content
TosCl	*p*-toluenesulfonyl chloride
UV-vis	ultraviolet-visible
δ	chemical shift in ppm and in-plane bending (scissoring)
η	viscosity
$[\eta]_{cuen}$	intrinsic viscosity determined in cupriethylenediamine
η_0	viscosity of solvent
η/η_0	relative viscosity
θ_c	contact angle
λ	wave length
ρ	in-plane bending (rocking)
ν	wave number and symmetrical/asymmetrical stretching

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1. General aspects of synthesis and structural features of ammonium group-containing cellulose esters

The use and modification of natural polymers is increasingly studied due to a their potential to improve our lifestyles. A fascinating biopolymer is cellulose, which is the most abundant carbohydrate biomass on Earth and has attracted widespread attention as a potential starting material for the preparation of smart materials [1-3]. The use of cellulose as a natural polymer has many advantages in comparison with traditional synthetic polymers: it is a renewable and biodegradable material, of low cost and low toxicity [4, 5]. The derivatization of cellulose to introduce specific functional groups is extremely interesting in order to tailor materials with advanced properties. Cationic functional groups such as quaternary ammonium groups increase cellulose hydrophilicity, ionic exchange capacity, adsorption capacity for anionic substances, etc. [6-8].

One disadvantageous aspect of cellulose is its insolubility in water and in most organic solvents, due to its supramolecular structure. Multi-component solvents as *N,N*-dimethylacetamide and *N*-methyl-2-pyrrolidone in presence of lithium chloride are efficiently used in homogeneous phase reactions. Ionic liquids like 1-butyl-3-methylimidazolium chloride are promising reaction media that allow the use of higher polymer concentrations. Ionic liquid solutions tend to be highly viscous and the addition of a co-solvent has been used to decrease their viscosity [9, 10].

Esterification of polysaccharides is a versatile transformation as it provides easy access to a variety of bio-based materials with valuable properties. Under alkaline and high-temperature conditions, the ester bonds are not stable. The controllable features of such exquisite compounds are the driving forces for their use in drug delivery [11], in release of dyes in washing-processes of fibers [12], in purification of drinking water [13], as catalysts in Knoevenagel reaction [14], etc. Novel esterifications via the ring-opening of lactams in presence of *p*-toluenesulfonyl chloride [15] and via conversion with (3-carboxypropyl)trimethylammonium chloride activated

with *N,N'*-carbonyldiimidazole are promising reactions to generate a novel ammonium group-containing cellulose esters. Cellulose derivatives functionalized with ester moieties displaying electropositive ammonium groups yield water-soluble cellulose derivatives imbued with interesting properties.

The aim of this work is to comprehend two different methods of esterification of cellulose. Dextran also is converted via the ring-opening reaction of lactams. Reactions performed at varied reactions conditions in multicomponent solvents and in ionic liquids are investigated. A detailed study of the structure characterization of the ammonium group-containing esters of polysaccharides by elemental analysis, FTIR- and NMR-spectroscopy is reported. A particular emphasis on the study of the charge behavior in aqueous solutions of varied pH values was given and its stability with increasing time was studied as well. In addition, preliminary studies on polyelectrolyte properties such as viscosity in the presence of salts and polyelectrolyte complex formation were also explored.

2. Literature review

2.1. Cellulose: occurrence, isolation and economic aspects

Cellulose is the natural polymer that makes up the living cells of all vegetation. It is the material at the center of the carbon cycle (from plants, algae, cyanobacteria, Fig.1) and the most abundant, biodegradable and renewable biopolymer on the planet.

It has been estimated that 10^{11} to 10^{12} tons of cellulose are synthesized annually by photosynthesis in a rather pure form (in seed hairs of the cotton plant, for instance), but mostly it is combined with lignin, hemicellulose and other substances (as pectin, waxes and mineral salts) in the cell wall of woody plants [17]. Further, cellulose-containig materials include agriculture residues, water plants, grasses, and other plant substances.

Highly crystalline cellulose is obtained from algae (*Valonia ventricosa* and *Chaetamorpha melagonicum*).

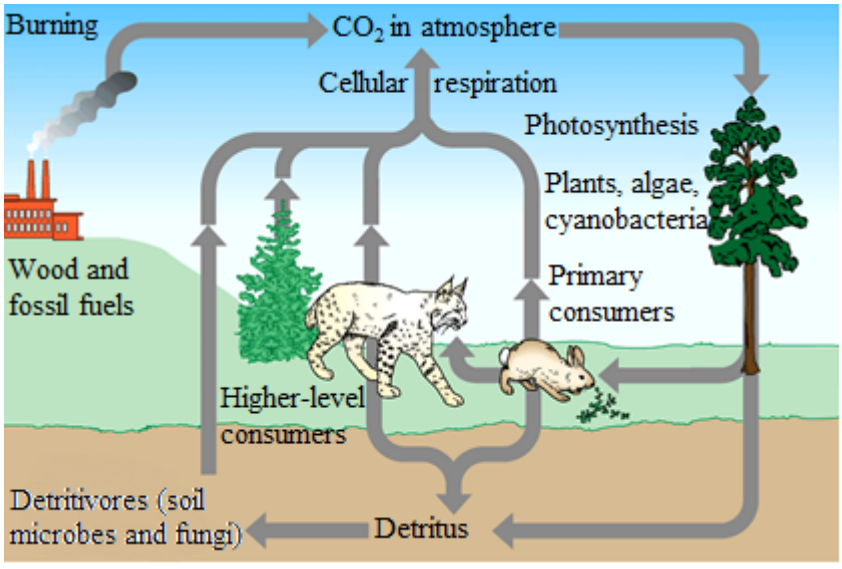


Fig. 1. Carbon cycle. The cell wall of plants, green algae and cyanobacteria are constituted by cellulose. Picture adapted from reference [16].

Moreover, pure cellulose can also be synthesized by acetic acid-producing bacteria as *Acetobacter xylinum* (bacterial cellulose) [18]. Cell walls are natural polymer composites constituted by cellulose, hemicellulose and lignin (Fig. 2) in a composition range of 40-44% of cellulose, 15-35% of hemicellulose and 18-25% of lignin for hardwoods [19]. *Eucalyptus globulus* containing 51.3% of cellulose is the richest source among various hardwood species. However, cotton is the best cellulose source since it contains 95% of the biopolymer [20].

Hemicellulose is a mixture of polysaccharides containing 500 to 3000 anhydroglucose units per polymer chain (xylose, mannose, galactose, rhamnose, and arabinose) and lignin is the product of the polymerization of various alcohols (paracoumaryl, sinapyl and coniferyl alcohols), responsible for the connection of the cellulose fibrils in woody plants. Cellulose is isolated of these compounds by large-scale chemical digestion processes (pulping) which dissolve them via a combined chemical transformations and cleavage. Hemicellulose is readily hydrolysed to sugars. Lignin is removed as lignosulfonic acid by the sulfite process, or as alkali or thiolignin by the Kraft and sulfate process respectively. Subsequent bleaching steps eliminate nearly all of the residual lignin.

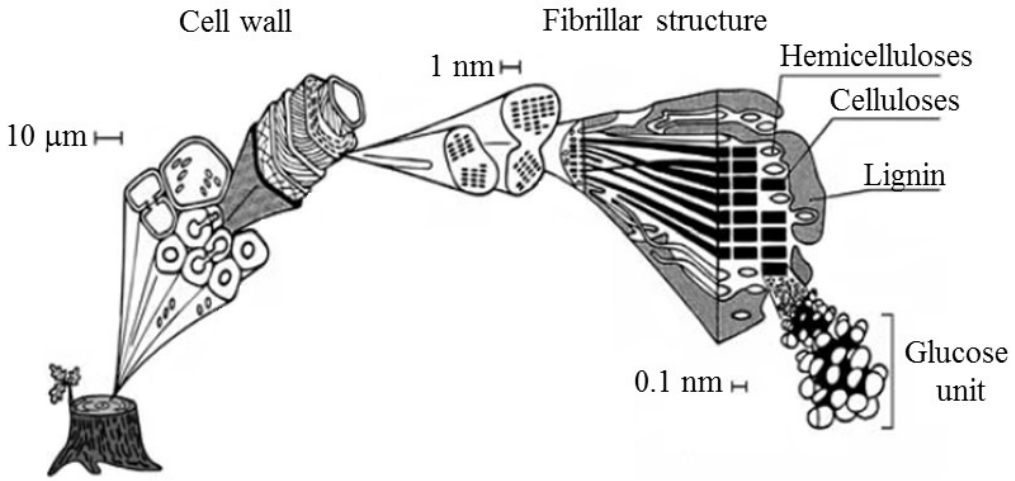


Fig. 2. Main composition of a cell wall: cellulose, lignin and hemicellulose. Ilustrated by Per Hoffmann [18].

Degradation of cellulose occurs and the glucose and oligosaccharides are removed by washing and filtration [4].

The application of cellulose covers fibers, paper, polymer, textile, and food industries [21-23]. Despite its abundance worldwide, cellulose cannot currently compete with petrochemistry on the grounds of costs. Several disadvantages of cellulose include its expensive production, its sensitivity to water, and its slow regeneration. A tree must have at least 30 years before it can be used for cellulose production. In this aspect, the use of non-wood lignocellulosics sources of short life cycle of agricultural cultivation of sisal [24] (73% in cellulose, an agave, the first harvest occurs 24 to 36 months after field planting and then continues to occur every 6–9 months), for instance, would favor the economic competitivity with respect to synthetic polymers [5].

2.2. Cellulose structure

In 1838, Anselme Payen (French chemist) was the first person to isolate cellulose from plants and determine its chemical formula [25]. The polymer structure of cellulose was determined by Hermann Staudinger in 1920 [26].

Regarding to its molecular structure, cellulose is a linear homopolymer consisting of β-(l→4)-glycosidic linked D-anhydroglucopyranose units (AGU). This linkage motif contrasts with that for α(1→4)-glycosidic bonds present in starch, glycogen, and other carbohydrates. With cellobiose as the basic unit, cellulose can be considered an isotatic polymer of cellobiose (Fig. 3). A hydrolytic treatment with aqueous acid at high temperature recovers D-glucose from cellulose (due to the degradation of the polymer backbone) [27].

The hydroxyl groups at both ends of the cellulose chain show different behavior. The C-1 end has reducing properties, while the glucose end group with a free C-4 hydroxyl group is non-

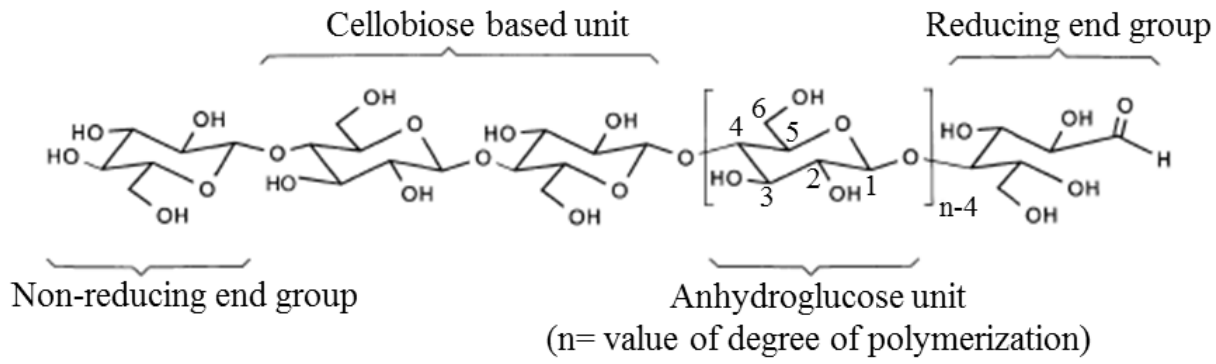


Fig. 3. Molecular structure of cellulose. Adapted from reference [4].

reducing. The bridging and the ring oxygen atom are predominantly involved in intra- and intermolecular interactions, mainly hydrogen bonds, and in degradation reactions. The multiple hydroxyl groups on the glucose from a chain form hydrogen bonds with oxygen atoms on the same or on a neighboring chain, holding the chains firmly together side-by-side and forming microfibrils with high tensile strength. This strength is responsible for the conformation and chain stiffness, and is important in cell walls where the microfibrils are meshed into a carbohydrate matrix, thereby conferring rigidity to plant cells. The anhydroglucose-ring has a $^4C_1$ chair conformation with the hydroxyl groups in equatorial and the hydrogens in axial positions. The molecular size of cellulose can be defined by the average degree of polymerization (DP). The DP results from the division of the average molecular mass ($\overline{M}$) by the molecular mass of the repeating unit AGU ($\overline{M}_{AGU}$) according to equation 1.

Eq. 1.

$$DP_n = \frac{\overline{M}_n}{\overline{M}_{AGU}}$$

The DP values of cellulose depend on the origin and pretreatment of the polymer. Isolated from native sources, it is always polydisperse due to the fact that it consists of a mixture of macromolecules with different chain lengths [4, 17, 18]. However, production of monodisperse cellulose beads has already been reported [28].

Regarding to its supramolecular structure, intra- and intermolecular hydrogen bonds in cellulose chains strongly influence the formation of aggregates of highly ordered structural entities (crystalline arrangements) due to their chemical constitution and their spatial conformation. It is referred to as a crystalline part since it exhibits a distinct X-ray diffraction pattern. Several different crystalline structures of cellulose are known, corresponding to the location of hydrogen bonds between and within strands [29]. The crystal structure is composed mostly by two groups of unit cells (monoclinic and triclinic space groups) [27]. The crystallinity of cellulose is the reason for its high stability by comparison with other polysaccharides. For instance, starch undergoes a crystalline to amorphous transition when heated beyond 60–70°C in water (as in cooking), cellulose requires a temperature of 320°C and pressure of 25 MPa to become amorphous in water [27, 30]. The less-ordered regions of cellulose structure are described as 'amorphous' and are the most accessible region of cellulose structure. The amorphous region is the first to be affected during the swelling process of cellulose via rupture of interfibrillar bonds. Intracrystalline swelling also occurs, increasing the lattice dimensions of the crystalline regions [27].

Microcrystalline celluloses like Avicel® (cellulose powder) are obtained from cotton linters by acid treatment, cellulase-catalyzed hydrolysis and via partial chain degradation. Thus, DP values between 150 and 300 are obtained [27, 31].

The anhydroglucose units (AGUs) of cellulose possess hydroxyl groups at C-2, C-3, and C-6 positions, capable of undergoing the typical reactions known for primary and secondary alcohols (etherification, esterification and oxidation, for instance). Hydroxyl groups at C-6 are less sterically hindered than the other hydroxyls, rendering it the most reactive position [32].

The derivatization of cellulose can be performed under heterogeneous or homogeneous reaction conditions. In industrial processes, heterogeneous conditions are almost exclusively used. The reaction initially involves the amorphous part and then the crystallites. At intermediate stages of

reaction, the cellulose derivatives have an uneven functionalisation pattern. They consist of regions with a high degree of substitution (DS) close to the low-substituted parts. As a consequence, it is difficult to control the structure of the product [33, 34]. Homogeneous derivatization possesses the advantage of the full availability of the hydroxyl groups which facilitates better control of the degree of substitution and a more uniform distribution of the functional groups along the backbone of the polymer [35].

2.3. Cellulose dissolution

The effective dissolution of cellulose is a prerequisite for its controlled functionalization. The most accepted concept for the dissolution of cellulose is that the intermolecular hydrogen bonds between the chains are broken and single polysaccharide chains are available in solution. The swelling of the polymer structure further facilitates the dissolution process [4]. Recently, it was hypothesized that cellulose would be soluble in amphiphilic solvents like ionic liquids, *N*-methylmorpholine-*N*-oxide and their cosolutes by weakening the hydrophobic interactions and hydrogen bonds [36]. Different solvents have been studied but only a few are able to dissolve the polymer. *N,N*-dimethylacetamide (DMAc) and its cyclic analogue *N*-methyl-2-pyrrolidone (NMP) in the presence of lithium chloride (LiCl) or lithium bromide (LiBr) are multi-component solvent systems as well as *N,N'*-dimethylformamide/LiCl, dimethylsulfoxide/LiCl, dimethylsulfoxide/tetrabutylammonium fluoride, N_2O_4/*N,N'*-dimethylformamide, *N,N*'-dimethylethyleneurea/LiCl and hexamethylphosphoric acid triamide/LiCl [37]. DMAc/LiCl and NMP/LiCl are often used for etherification and acylation reactions. In 1985, McCormick *et al.* [38] proposed that it would be possible to dissolve cellulose in these two solvent systems via complex-formation (as depicted in Fig. 4). A detailed study of the theory was presented in 1990 by Dawsey and McCormick [32]: the hydroxyl protons of the AGU are associated with

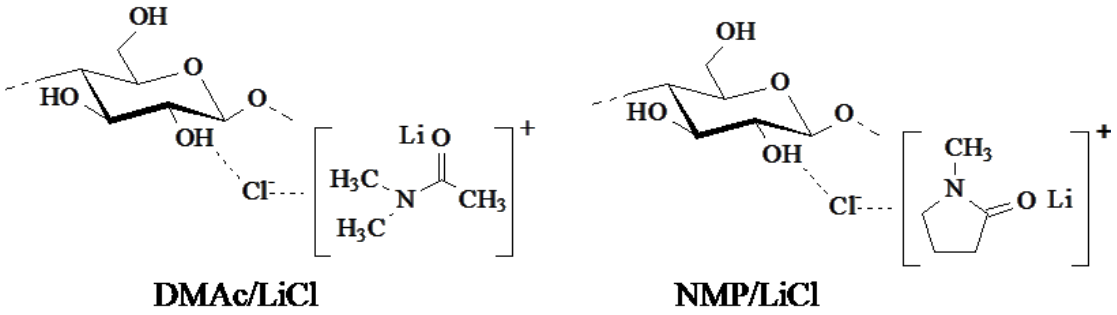


Fig. 4. Structure of the cellulose/DMAc/LiCl and cellulose/NMP/LiCl complexes proposed by McCormick C. L. *et al.* [38]. Figure adapted.

the chlorine anion by hydrogen bonding. The chlorine ion is associated with a $Li^{+}(DMAc)_x$ and $Li^{+}(NMP)_x$ macrocations. The resulting charge repulsion would allow further solvent penetration into the polymer structure. There are different points of view about the mechanism of cellulose dissolution and it has not yet been conclusively determined [39]. However, the use of efficient solvents is restricted to lab-scale syntheses due to their toxicity, high cost and poor recyclability [37].

Ionic liquids (ILs), a group of imidazolium, pyridinium or halide/halogenoaluminate salts existing as liquids at relatively low temperatures (< 100°C), might overcome these limitations. They have many attractive properties, such as negligible vapor pressures, chemical and thermal stability, and non-flammability. The acidity, viscosity and melting point of these compounds can be adjusted by changing the alkyl substituents and the imidazolium/pyridinium [40] and/or the halide/halogenoaluminate ratios [41].

ILs, represent a new class of 'green solvents' that are a promising single-component solvents for natural polymers, which have been found to be more compatible with the environment since their recovery/recycling is possible [10, 42]. Cellulose can be dissolved in rather high concentrations (up to 15-20%) within short times (1-12 h) without any pretreatment [43, 44]. Microwave heating can significantly accelerate the dissolution process [45]. The solubility of different substances in imidazolium ionic liquids depends mainly on polarity and hydrogen bonding

ability. For instance, the presence of chloride atoms in 1-butyl-3-methylimidazolium chloride (BMIMCl, Fig. 5) and in 1-allyl-3-methylimidazolium chloride is assumed to be highly effective in breaking the extensive hydrogen-bonding network present in cellulose, which makes these ILs very useful as reaction media. The presence of water in BMIMCl greatly decreases the solubility of cellulose through competitively hydrogen-bonding its microfibrils [43, 46]. The use of 1-ethyl-3-methylimidazolium acetate (liquid at room temperature) was found to be not appropriate as reaction medium of cellulose acylation, tritylation and tosylation since it may undergo several side reactions [47].

The advantages of ILs as solvents for polysaccharides are combined with some limitations, such as high viscosity of the IL and the corresponding cellulose solution, and limited miscibility of the IL with hydrophobic reagents and cellulose derivatives. The high viscosity may lead to a non-uniform and irreproducible reaction product. These drawbacks can be minimized by adding co-solvents to cellulose/IL solutions. Recently, Gericke *et al.* [9] studied in detail the use of varied co-solvents in ternary systems (cellulose/IL/co-solvent) and identified DMSO as a promising co-solvent.

DMAc/LiCl, NMP/LiCl and BMIMCl are useful reaction media for the conversion of cellulose. Among all possible transformations of alcohols, esterification and etherification of polysaccharides represent the most versatile reactions as they provide easy access to a variety of bio-based materials with valuable properties.

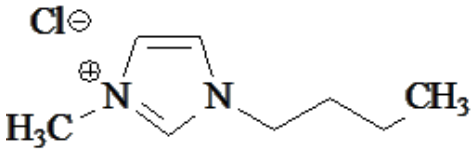


Fig. 5. Structure of the ionic liquid 1-butyl-3-methylimidazolium chloride, melting point = 73°C.

2.4. Homogeneous esterification of cellulose

Cellulose acetate, propionate, butyrate and phthalate are cellulose esters that have been industrially employed. They are used as filters in cigarettes, films, fibers, membranes, tubes, utensils, eyewear, fashion accessories, pens, brushes and toys, among others [48, 49]. The hydroxyl groups of cellulose can be esterified using typical reagents, such as organic acids under water-free conditions, or reactive acid derivatives (carboxylic acid halides and -anhydrides) and reaction conditions applied in low-molecular organic chemistry. Several derivatization agents are used in heterogeneous esterification reactions [34]. After the development of new solvent systems for cellulose, novel products were created under homogeneous reaction conditions with an excellent control of the degree of substitution (DS).

In this context, homogeneous esterifications by ring-opening reactions of lactams and *in situ* activation of carboxylic acids are able to generate cationic and water-soluble cellulose esters.

Esterification by ring-opening reaction of lactams and of cyclic carboxylic acid derivatives

These esterification reactions work via the cleavage of lactone or lactam rings with subsequent ester formation along the polymer backbone. For instance, non-ionic mixed cellulose esters containing acetoacetate- and other carboxylic acids were obtained by the ring-opening of diketenes in the presence of carboxylic acid anhydrides forming an acetylketene as a reactive intermediate. The reactions occurred in 30 min at 110°C in DMAc/LiCl or in NMP/LiCl and water-soluble cellulose esters with DS up to 2.91 were obtained [50].

Extending the concepts for other polysaccharides, the ring-opening of ε-caprolactone and D,L-lactide using tin octanoate as catalyst has been studied to obtain non-ionic esters. Pullulan-6-hydroxycaproate esters with a DS value between 0.10 and 0.5 were generated in DMSO with ε-

caprolactone [51]. Polylactide-grafted dextran copolymers were obtained from silylated dextran with D,L-lactide dissolved in toluene after a reaction time of 20 h at 100°C [52]. Conversion of 99% of the polymer was obtained. Hydroxyethyl chitosan-g-poly(D,L-lactide) was synthesized from hydroxyethyl chitosan with D,L-lactide by a microwave-assisted reaction at 140°C (in 20 min) [53]. A product with 229% of grafting was obtained.

Ring-opening reaction of *N*-methyl-2-pyrrolidone

Cationic cellulose esters can be prepared via the ring-opening of *N*-methyl-2-pyrrolidone (NMP) in NMP/LiCl. A reactive intermediate of a Vielsmeier-Haack-type reaction is formed by the interaction of NMP with *p*-toluenesulfonyl chloride (TosCl). Figure 6 summarizes all the possible products formed. The mechanism is based on the *in situ* generation of the O-(*p*-toluenesulphonyl)-*N*-methyl-2-pyrrolidinium salt **I** (Fig. 7) and its reaction with the hydroxyl groups of the anydro-glucose unit, depending on the conditions utilized. Using pyridine (a weak organic base, pK_a 5.25), the reaction with the polysaccharide yields a reactive *N*-methyl-2-pyrrolidinium salt **II**. **II** can form the acetylated polysaccharide **III** after aqueous workup (Fig 7, also shown in Fig. 6a), or regenerate cellulose and NMP (Fig. 6b), or generate deoxychloro compounds at high temperatures (Fig. 6c) [54]. In addition, stronger bases such as triethylamine (pKa 10.65) can react with **I**, yielding a polysaccharide sulphonic acid ester (Fig. 6d) [15, 39]. The competitive formation of the deoxychloro cellulose (Fig. 6c) and cellulose *p*-toluenesulfonate (Fig. 6d) are undesirable side-reactions and take place when the starting material (cellulose) is dissolved in DMAc/LiCl [54].

Interestingly, in a Vielsmeier-Haack-type reaction, cellulose has been acylated with ε-caprolactone in presence of triethylamine in DMAc/LiCl at 80°C for 18 h yielding a cationic DMSO-soluble cellulose-6-hydroxycaproate with a DS of 0.8 [55]. Moreover, the conversion of

cellulose with ε-caprolactone, *N*-methyl-ε-caprolactone and *N*-methyl-2-piperidone dissolved in the ionic liquid BMIMCl using DMAc as co-solvent have also been studied. Ammonium group-containing cellulose esters of different alkyl chain lengths were obtained by applying TosCl as activator after 5 h of reaction time at room temperature. Products with DS of 1.17 were yielded using a molar ratio of 1:2:2 (AGU:TosCl:lactam) [56].

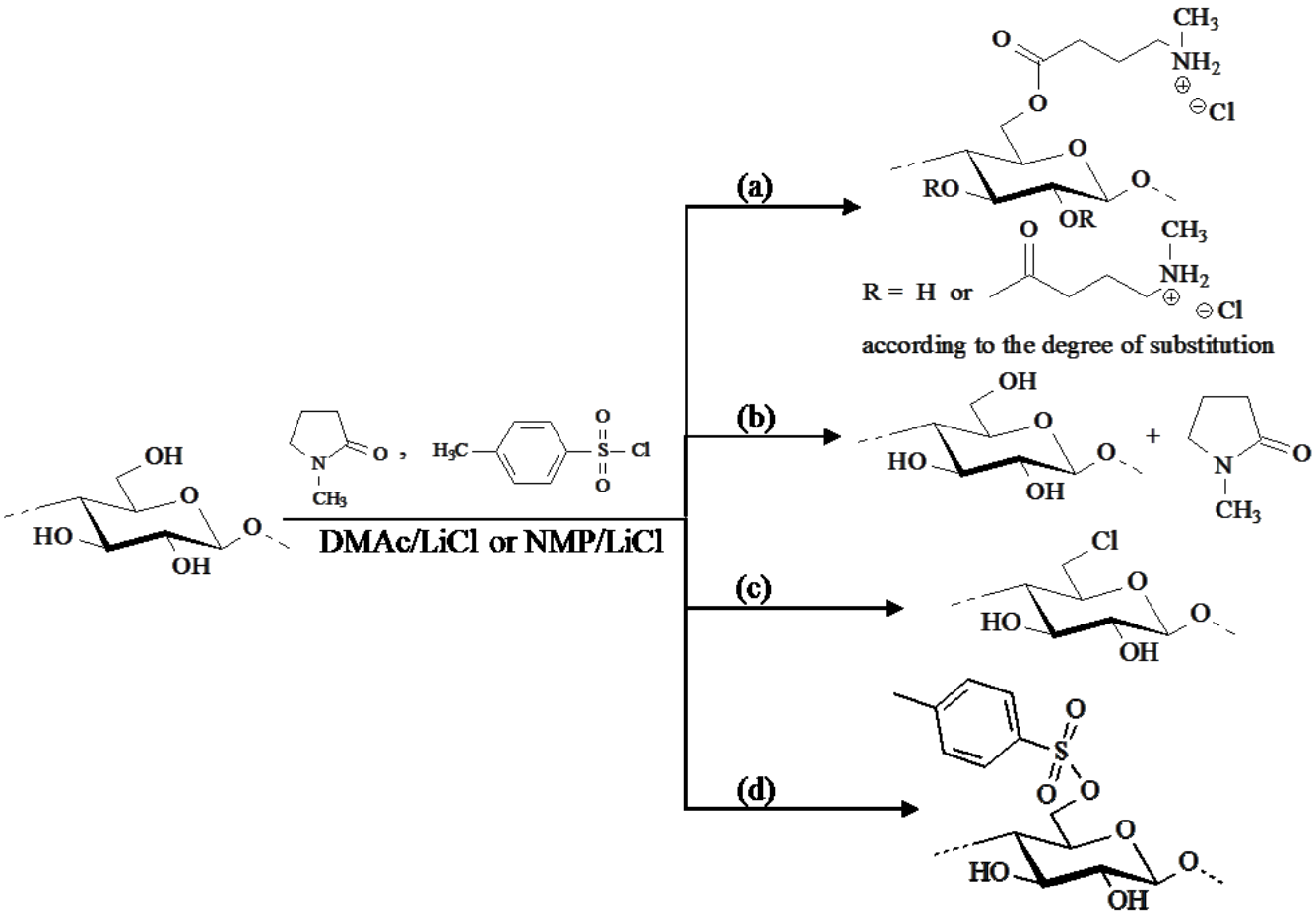


Fig. 6. Products generated from the esterification of cellulose with *N*-methyl-2-pyrrolidone in the presence of *p*-toluenesulfonyl chloride: cellulose-4-*N*-[methylammonium]butyrate chloride (a), regeneration of the reagents (b, reaction not occurred), deoxychloro cellulose (c, undesirable side-product), and cellulose *p*-toluenesulfonate (d, undesireable side-product).

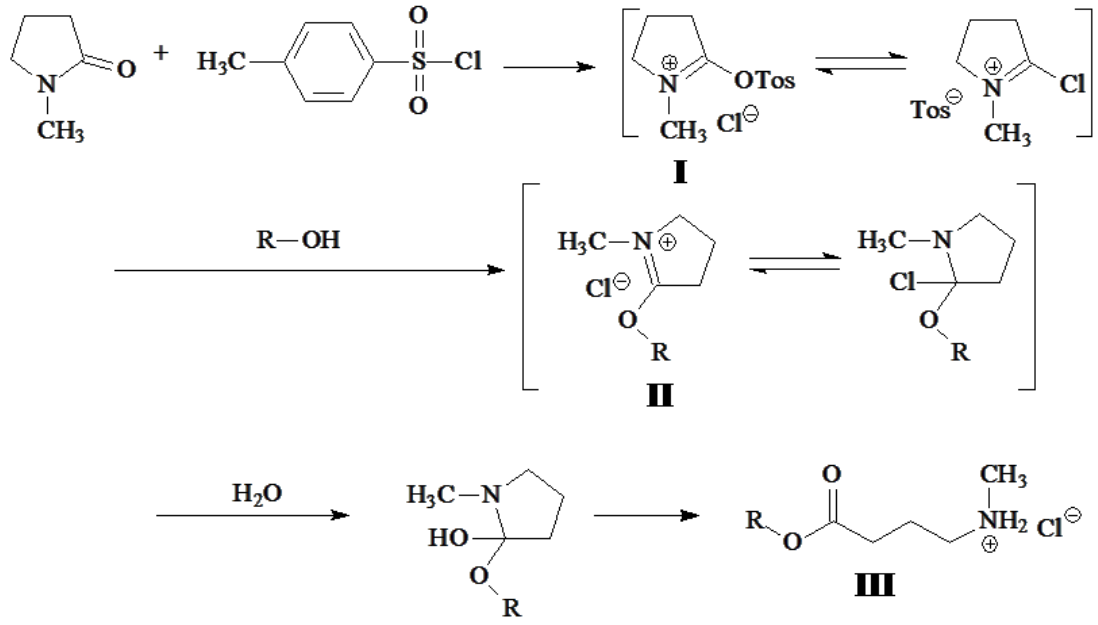


Fig. 7. Schematic representation of the conversion of alcohols (R-OH) with *N*-methyl-2-pyrrolidone in the presence of *p*-toluenesulphonyl chloride to form an ammonium group-containing carboxylic acid esters. Adapted from reference [39].

Esterification by *in situ* activation of carboxylic acids

Carboxylic acids can be *in situ* activated by reaction with another compound leading to a reactive intermediate. The carboxylic acid derivative may be formed prior to the reaction with the polysaccharide or *in situ* activation is carried out directly in the biopolymer solution [39].

Homogeneous esterification (in DMF/LiCl or in DMAc/LiCl) of cellulose and other polysaccharides can be performed by activation of carboxylic acids with TosCl or methanesulfonyl chloride. Several steps are required for the purification of the products from TosCl impurities compared to cellulose esters generated by the use of another activating agent [57, 58]. In peptide or protein chemistry, dialkylcarbodiimides are commonly used as activating agents for complex carboxylic acids despite their toxicity (LD_{50} 71 mg/kg) [59]. The use of iminium chlorides in the *in situ* activation of carboxylic acids was effective in esterification

using long-chain aliphatic acids (stearic acid and palmitic acid). They are formed by conversion of *N,N'*-dimethylformamide with a chlorinating agent (oxalyl chloride) and subsequent reaction with the acid [60].

The use of *N,N'*-carbonyldiimidazole (CDI) in the *in situ* activation of the carboxylic acids facilitates the purification of the cellulose esters since imidazole is soluble in a broad variety of solvents and can be easily removed. This esterification method has been applied in several studies [39].

Esterification of cellulose with the following carboxylic acids was performed by *in situ* activation using CDI: (−)-menthyloxyacetic acid, 3-(2-furyl)-acrylcarboxylic acid, furan-2-carboxylic acid, 4'-carboxybenzo-18-crown-6, and carboxymethyl-*β*-cyclodextrin [61]. Cellulose was dissolved in DMSO/TBAF or in DMAc/LiCl. Cellulose esters containing different moieties were generated. Water-insoluble cellulose furoates were synthesized in the ionic liquid BMIMCl by *in situ* activation of 2-furancarboxylic acid with CDI [62]. Dorn *et al.* [63] proved that esterifications performed in ionic liquids (BMIMCl, EMIMCl and AMIMCl) are more efficient than those carried out in DMAc/LiCl. The experiments were performed using the reactive 3,6,9-trioxadecanoic acid imidazolide and 3,6-dioxaheptanoic acid imidazolide, yielding water-soluble products. Recently, water-soluble ammonium group-containing dextran esters were synthesized by *in situ* activation of amino- and ammonium-acids with CDI. Dextran was dissolved in DMF/LiCl and a product with DS of 0.22 (Fig. 8) was obtained with (3-carboxypropyl)trimethyl

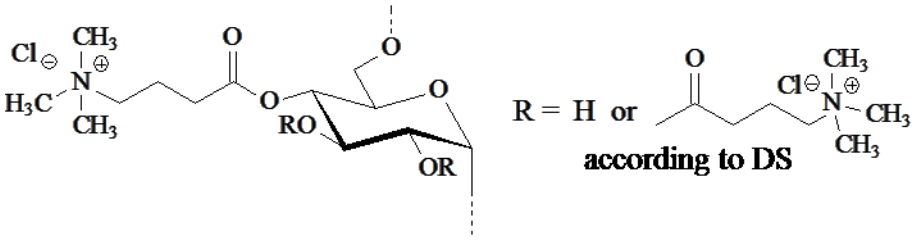


Fig. 8. Structure of ammonium group-containing dextran ester. DS, degree of substitution. Adapted from reference [11].

ammonium imidazolide at 50°C after 18.5 h of reaction time. Such products were tested in drug delivery assays [11].

Cationic ammmonium group-containing polysaccharide esters cannot be easily obtained. For example, Porjavadi *et al.* [14] reported a three step synthesis via phenyl carbonate cellulose (Fig. 9a) and *N,N*-diethylaminopropyl cellulose carbamate (Fig. 9b) in order to obtain a quaternary ammonium cellulose ester (Fig. 9c).

The water-solubility in cationic derivatives of polysaccharides is related to the large number of small counterions, which contribute strongly to the entropy. In addition, the electrostatic repulsion between charged polymer backbones may also contribute to dissolution, but Coulombic interactions are much less significant and can also have the opposite effect [64].

2.5. Cationic polyelectrolytes based on polysaccharides

The introduction of ionic moieties in cellulose structure by esterification reaction is an interesting path to obtain water-soluble charged polymers.

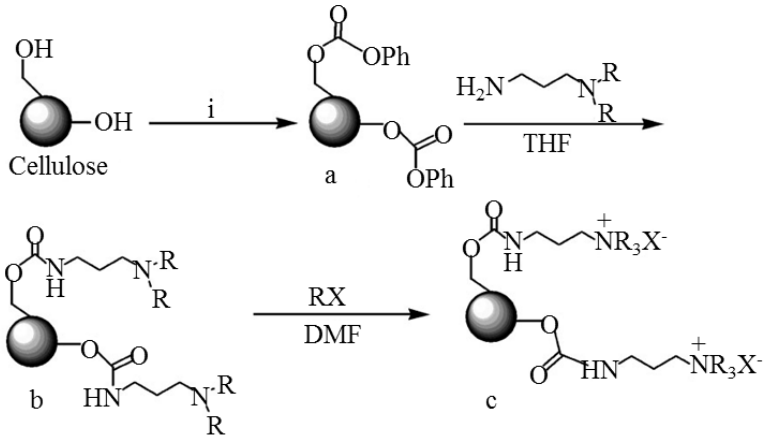


Fig. 9. Synthesis of nitrogen-functionalized cellulose derivatives using phenyl carbonate as precursor. Reagents and conditions: i = phenyl chloroformate/DMAc/LiCl/ pyridine; R = Et; X = Br. A, b and c are isolated products. Adapted from reference [14].

The term 'polyelectrolyte' is employed for polymer systems consisting of a 'macroion', that is a macromolecule carrying covalently bond anionic or cationic groups, and low-molecular counterions. Nearly all the cellulose-based polyelectrolytes known today are anionic (mainly, carboxymethylcellulose). The properties of polyelectrolyte solutions such as viscosity are strongly dependent on the ionic strength of the aqueous medium. Changes in solution viscosity with increasing ionic strength are mainly caused by an electrostatic shielding of the electric charges of the macroion with the later increasingly approaching the behavior of a 'normal' uncharged macromolecule. Besides the ionic strength, the pH of the medium affects strongly the properties of weak polyelectrolytes such as amine moieties in solution, as the pH determines the degree of dissociation of a given ionic group and thus the actual charge density of the polyelectrolyte in question. These characteristics are very important to the study of polyelectrolyte interactions and techniques such as polyelectrolyte titration and potentiometric titration are suitable to determine the charge behavior. In addition, the interaction of the macroions with oppositely charged species is predominantly governed by the action of electrostatic forces, hydrogen bonds and hydrophobic forces, however, the Coulombic forces play the major role [65].

Water-soluble ammonium group-containing cellulose derivatives are of great technological interest. As a result of the presence of cationic groups, such cellulose derivatives exhibit increased substantivity to a variety of substrates and, consequently applications in many areas might be found. Quaternized nitrogen-containing cellulose ethers like grafted copolymer of a *N,N*-dialkyldiallyl ammonium halide on a substrate of cellulose were developed, and have been used in conditioning human hair due to their adsorption by the proteinaceous structure of hair. The compounds have good compatibility, gloss, adhesion to hair, resistance to static generation and superior holding properties even under conditions of high humidity [66, 67].

Some compounds such as *N,N*-dimethylaminoethylcarbamoyl cellulose showed antimicrobial activity, which is useful for the preservation of cosmetic products and may also be used as

thickeners or conditioners [68, 69]. These compounds have also been used to determine the change in surface tension, foamability and foam stability resulting from the complexation with sodium dodecyl sulfate in aqueous solutions [70, 71].

Using water-soluble quaternized hydroxyethyl cellulose derivatives, water-sensitive shale formations containing large amounts of clay minerals were stabilized (oilfield industry). The clay-hydration is more effectively inhibited by the cationic cellulose derivative than using conventional anionic or non-ionic ones due to its affinity for negatively charged clay surfaces [72]. The performance of these compounds is strongly dependent on their cationicity, intrinsic viscosity and solution concentration [73]. It is also of considerable interest to note that cellulose derivatives with amine or ammonium anchor groups can find application in chemical library generation [74]. Recently, cationic ammonium group-containing cellulose esters were found to be a green and selective catalyst in Knoevenagel reaction [14]. Under alkaline and high-temperature conditions, the ester bonds are not stable and break. The controllable features of such exquisite compounds are the driving forces for their use in drug delivery, in the release of dyes during the washing-process of fibers, and in the purification of drinking water [11-13]. Cellulose esters are very important because their glass-transition temperatures, Tg, and melting temperatures, Tm, are relatively low. This allows their processing by injection molding, and casting from the melt, a problem that has limited the applications of cellulose [34]. Despite the large number of publications, investigations correlating quantitatively end-use properties to reaction conditions and structural changes resulting therefrom are comparatively scarce.

2.6. The importance of cellulose ethers

Cellulose etherification is a very important branch of commercial cellulose derivatization, generating products that, in solution, increase the viscosity of the medium. Therefore, most of cellulose ethers are used as thickeners. In 1905, the first cellulose ether was synthesized: methyl

cellulose, widely used nowadays in food and cosmetic products and also as a treatment of constipation since it is not toxic and not allergenic. After 1920, the synthesis of carboxymethyl cellulose and hydroxyethyl cellulose were described [75]. Carboxymethyl cellulose (CMC) is an anionic cellulose derivative which contains carboxymethyl groups (-CH_2-COOH) in a sodium salt form (-CH_2-COONa), and it is used in emulsions of ice cream, toothpaste, laxatives, diet pills, water-based paints, textile sizing and paper products. Interestingly, due to its negative charge, several studies using this compound as a polyanion have been carried out as, for instance, the development of eco-friendly flocculants by grafting of CMC in polyacrylamides [76, 77].

Also commercially important cellulose ethers are hydroxyethyl- and hydroxypropyl cellulose. Depending on the degree of substitution (DS) and on the molecular substitution (MS > 1), they can be water-soluble compounds. The DS denotes the average number of cellulosic hydroxyl groups per AGU involved in the reaction and the MS denotes the average number of alkylene oxide molecules added per AGU. Usually, hydroxyalkyl ethers of cellulose are gelling agents, thickeners or binders used in cleaning solutions, in capsule formation and in ophthalmic solutions [78, 79]. Usually, hydroxyethyl ethers of cellulose are industrially synthesized in a heterogeneous medium via reaction of cellulose with ethylene oxide (Fig. 10). Aqueous alkali metal hydroxides are used as catalysts. Oxyalkylene chains of varying lengths are yielded due to

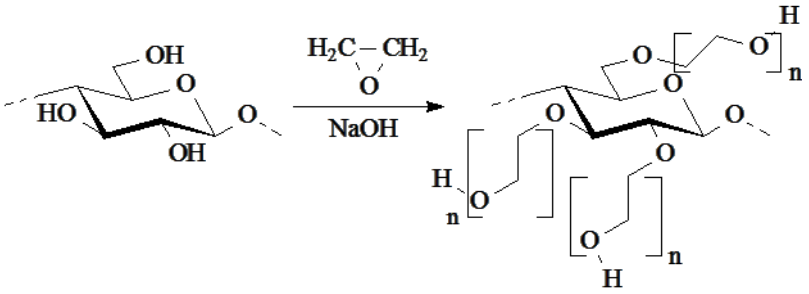


Fig. 10. Hydroxyethylation of cellulose in aqueous alkaline medium. Adapted from the reference [80].

the fact that the hydroxyalkylation proceeds further at the newly formed hydroxyl groups [80, 81].

Homogeneous synthesis of hydroxyethyl cellulose using chlorohydrin/acetic acid dissolved in NaOH/urea/water (at -12°C) in a lab-scale was reported [82]. Hydroxyethyl cellulose is an interesting compound to be used as starting material for subsequent esterification reaction and generation of novel macromolecules since they possess hydroxyl groups in its structures capable of undergoing the typical reactions for primary alcohols.

Among the importance in to generate novel ammonium group-containing cellulose esters, another remarkable polysaccharide is dextran. The conversion of dextran and subsequent formation of smart products of interesting properties could be a significative object for advances in the medical area.

2.7. Dextran

Dextran, produced by numerous strains of bacteria (*Leuconostoc mesenteroides* and *Streptococcus mutans*), is a family of neutral polysaccharides consisting of a α-1,6-linked D-anhydroglucose unit main chain (Fig. 11), which is branched by an α-1,2-linked anhydroglucose unit. Varying proportions of linkages and branches are found, depending on the bacteria used for the synthesis [83]. It is produced on an industrial scale from sucrose by bacterial enzymes (*Leuconostoc mesenteroides*) with a molar mass of about 10^5 g/mol. The polysaccharide contains a low level (approximately 5%) of randomly distributed α-(1→3) branched linkages in its structure [84]. They are generally soluble in water and in solvents such as formamide and glycerol.

Dextran is biocompatible, biodegradable, non-immunogenic and non-antigenic which

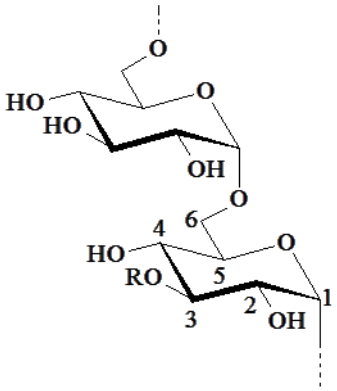


Fig.11. Structure of dextran obtained from *Leuconostoc mesenteroides*. R = predominately H and 5% glucose or α-(1→6) linked glucopyranosyl-α-D-glucopyranoside (not shown).

qualifies this polysaccharide as a physiologically harmless biopolymer [85].

It is used medicinally as an antithrombotic agent (anti-platelet) decreasing the blood viscosity, and as a blood volume expander in anemia [86]. It can be metabolized after intra-arterial and intravenous administration and it also increases the blood sugar levels. Moreover, dextran can be depolymerized by different α-1-glycosidases (dextranases) which occurs in the liver, spleen, kidney, and the lower part of the gastrointestinal tract. Due to the great importance of dextran in the medical arena, derivatization of this biopolymer with cationically charged ester moieties is an interesting way to impart further properties and hence to extend its field of application. The biopolymer possesses hydroxyl groups at C-2, C-3 and C-4 positions, capable of undergoing the typical reactions known for secondary alcohols. The OH groups of the AGUs can be esterified or etherified in a conventional manner under homogeneous reaction conditions, preferentially in an aqueous medium [65, 87]. Dextran-based polyelectrolytes of practical relevance are dextran sulfate, obtained by reaction of dextran with $ClSO_3H$ in pyridine and subsequent neutralization of the acidic half-ester to the stable sodium salt, and diethylaminoethyl dextran, prepared by aminoalkylation with β-chloroethyldiethylamine hydrochloride in the presence of hydroxide ions [88, 89]. Different methods for dextran sulfation are found in the literature [87]. The synthesis of dextran-4-[*N,N,N*-trimethylammonium]butyrate chloride was reported [11]. The derivatization of

dextran leads to a polyelectrolyte with an improved water solubility, giving aqueous solutions with defined rheological properties, which are valuable as viscosity-regulating agents. Cationically modified dextrans are interesting compounds due to their potential for spontaneously forming nanocomplexes via electrostatic interactions. They could be able to reduce the electrostatic repulsion between DNA and cell surfaces [90], neutralizing the negative charges, and are therefore promising macromolecules for gene delivery studies [11].

3. Results and Discussion

3.1. Synthesis and characterization of novel ammonium group-containing cellulose-, hydroxyethyl cellulose- and dextran esters

Cationic ammonium acid esters based on cellulose, hydroxyethyl cellulose and dextran were synthesized via ring-opening reaction of lactam. Esterification of cellulose with carboxylic acids facilitates the synthesis of quaternary ammonium group-containing cellulose esters.

3.1.1. Ring-opening reactions of *N*-methyl-2-pyrrolidone

4-[*N*-Methylammonium]butyrate chlorides from cellulose, hydroxyethyl cellulose and dextran were synthesized under homogeneous conditions by conversion of the biopolymer with *N*-methyl-2-pyrrolidone (NMP) in presence of *p*-toluenesulphonyl chloride (TosCl).

3.1.1.1. Synthesis and characterization of cellulose-4-[*N*-methylammonium]butyrate chloride

Ring-opening of *N*-methyl-pyrrolidone was used for the conversion of cellulose to cellulose-4-[*N*-methylammonium]butyrate chlorides (Fig. 12).

In accordance with the procedure described by McCormick *et al.* [15], a cellulose solution in NMP/LiCl was reacted with 11 mol TosCl and 11 mol pyridine per mol AGU yielding a cellulose ester with a DS of 0.78 (sample **4**, Table 1). Decreasing the molar ratio to 1:5.5:5.5 (AGU:TosCl:pyridine) afforded a sample with a slightly decreased DS of 0.62 (sample **6**) at a comparable reaction time and temperature.

It could also be demonstrated that pyridine is not necessary to accomplish the reaction using the same molar ratio (1:5.5:5.5, AGU:TosCl:pyridine); a sample with a comparable DS of 0.77

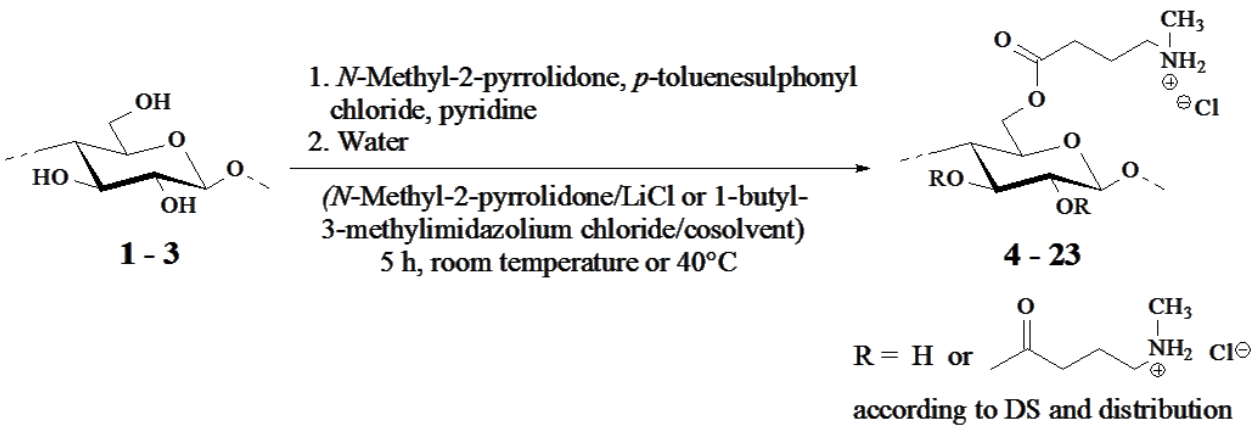


Fig. 12. Conversion of cellulose with *N*-methyl-2-pyrrolidone in presence of *p*-toluenesulphonyl chloride to obtain cellulose-4-[*N*-methylammonium]butyrate chloride. Degree of substitution, DS.

(sample **7**) was obtained in the absence of pyridine. Reaction for a short time (2 h) at room temperature (RT) led to a product with lower DS of 0.31 (sample **5**), on one hand. On the other hand, a sample possessing a DS of 1.01 (sample **11**) could be obtained after a reaction time of 16 h. Regarding the reaction temperature, it was found that no higher DS values were achieved at comparable molar ratio and reaction time. A sample with a DS of 0.91 (sample **13**) was prepared after 16 h at 40°C. Further increase of the reaction temperature to 60°C does not yield products of higher DS. On the contrary, the DS was decreased (DS of 0.24, sample **14**) and a high chlorine content of 12% indicated undesired formation of deoxychloro moieties. A certain amount of sulphur content caused by tosylate anions was determined by elemental analysis in some samples. The anions were easily exchanged by Cl^- using an ionic exchange resin.

Different cellulose types (microcrystalline cellulose, **1**, spruce sulphite pulp, **2**, and cotton linters, **3**, Table 1) were included in the studies in order to vary the DP of the products. The DP of the cellulose used as starting material does not play a significant role.

However, the higher DP, the higher viscosity of the reaction mixture and, hence, mixing and temperature control of the mixture become more difficult. Wood pulp (**2**, DP_{cuen} 912) and cotton

Table 1. Conditions for and results of the conversion of cellulose dissolved in *N*-methyl-2-pyrrolidone/LiCl or 1-butyl-3-methylimidazolium chloride with *N*-methyl-2-pyrrolidone in the presence of *p*-toluenesulphonyl chloride.

Conditions							Results						
Cellulose[a]	Solvent	Molar ratio[b]			Temp. (°C)[c]	Time (h)	Sample	Elemental analysis (%)		DS[d]	DP_n[f,g]	DP_w	PDI
		NMP	TosCl	Py				N	Cl[f]				
1	NMP/LiCl	excess	11	11	RT	5	**4**	4.07	10.35	0.78	78	284	3.66
1	NMP/LiCl	excess	5.5	5.5	RT	2	**5**	2.14	5.21	0.31	99	614	6.18
1	NMP/LiCl	excess	5.5	5.5	RT	5	**6**	3.52	9.86	0.62	65	226	3.50
1	NMP/LiCl	excess	5.5	-	RT	5	**7**	4.06	9.69	0.77	81	392	4.84
2	NMP/LiCl	excess	5.5	5.5	RT	5	**8**	4.29	11.34	0.85	155	588	3.79
3	NMP/LiCl	excess	5.5	5.5	RT	5	**9**	4.32	11.39	0.86	415	1188	2.86
1	NMP/LiCl	excess	5.5	5.5	RT	16	**10**	4.71	11.79	1.04	72	154	2.14
1	NMP/LiCl	excess	5.5	5.5	RT	16	**11**	4.73	12.06	1.01	109	411	3.78
1	NMP/LiCl	excess	5.5	5.5	40	5	**12**	4.40	11.92	0.89	78	365	4.68
1	NMP/LiCl	excess	5.5	5.5	40	16	**13**	4.46	14.21	0.91	84	397	4.70
1	NMP/LiCl	excess	5.5	5.5	60	5	**14**	1.75	12.54	0.24	101	288	2.85
1	BMIMCl	2	2	-	RT	2	**15**	0.93	2.41	0.12[e]	n.d.	n.d.	n.d.
1	BMIMCl	2	2	-	RT	5	**16**	2.61	7.43	0.40	54	168	3.11
1	BMIMCl	2	2	2	RT	5	**17**	3.39	8.57	0.56	65	223	3.40

Table 1. (cont.)

Conditions							Results						
Cellulose[a]	Solvent	Molar ratio[b]			Temp. (°C)[c]	Time (h)	Sample	Elemental analysis (%)		DS[d]	DP_n[f,g]	DP_w	PDI
		NMP	TosCl	Py				N	Cl[f]				
1	BMIMCl	2	2	-	40	2	**18**	2.84	8.13	0.45	44	148	3.40
1	BMIMCl	2	2	-	40	5	**19**	3.56	10.57	0.63	36	96	2.71
1	BMIMCl	2	2	-	40	16	**20**	3.55	11.90	0.63	33	88	2.67
1	BMIMCl	2	2	-	RT	16	**21**	3.26	8.70	0.55	48	165	3.41
1	BMIMCl/NMP	2	2	-	RT	2	**22**	2.13	5.94	0.31	67	203	3.02
1	BMIMCl/NMP	2	2	-	RT	5	**23**	3.53	9.18	0.62	74	318	4.32

[a] **1**: Microcrystalline cellulose (DP_{cuen} 156, DP_n 99, DP_w 339, PDI 3.42), **2**: spruce sulphite pulp (DP_{cuen} 912, DP_n 905, DP_w 12658, PDI 13.99), **3**: cotton linters (DP_{cuen} 1795, DP_n 1702, DP_w 15430, PDI 9.06). DP_n and DP_w were determined by SEC after percarbanilation of the starting materials; [b] molar ratio anhydroglucose unit: *N*-methyl-2-pyrrolidone:*p*-toluenesulphonyl chloride: pyridine; [c] room temperature (RT); [d] degree of substitution (DS) calculated from the nitrogen content; [e] this sample was water-insoluble; [f] not determined (n.d.); [g] degree of polymerization (DP) calculated from $\overline{M}_n$ and $\overline{M}_w$.

linters (**3**, DP_{cuen} 1714) could be converted to water-soluble products with reasonable DS of 0.85 (sample **8**) and 0.86 (sample **9**), respectively, with 5.5 mol of TosCl and 5.5 mol of pyridine per mol of AGU after 5 h at RT.

In a further series of experiments, the ionic liquid 1-butyl-3-methylimidazolium chloride was used as single component solvent for cellulose. The conversion of microcrystalline cellulose **1** (DP_{cuen} 156) with 2 mol NMP and 2 mol TosCl for 2 h at room temperature afforded a water-insoluble product with DS of 0.12 (sample **15**). Prolongation of the reaction time from 2 to 5 h yielded a product with DS of 0.40 (sample **16**). The addition of an equimolar amount of pyridine related to TosCl and NMP increased the DS slightly (DS of 0.56, sample **17**). The increase of the reaction temperature from room temperature to 40°C led to an increase of the DS from 0.12 (sample **15**) to 0.45 (sample **18**), yielding a water-soluble product. Furthermore, a higher DS of 0.63 (sample **19**) could be achieved by prolonging the reaction time from 2 to 5 h at both comparable molar ratio (1:2:2, AGU:NMP:TosCl) and reaction temperature. It appeared that the reaction is complete after 5 h because the product isolated after 16 h of reaction time at 40°C possessed a DS of 0.63, too (sample **20**). Reaction for 16 h at room temperature gave a slightly lower DS of 0.55 (sample **21**). The synthesized cellulose-4-[*N*-methylammonium]butyrate chlorides except sample **15** are readily soluble in water and DMSO.

Cellulose solutions in ionic liquids are highly viscous. This drawback could be circumvented by the increase of the temperature or by the addition of a co-solvent, for instance, NMP (10 mL per 5 g of cellulose solution), which is also the reagent.

The DP of the starting polymers and the products was determined in order to compare both reaction media in terms of polymer degradation (Table 1). Polymer degradation was observed in many cases, particularly those with increased reaction times and temperatures. It was found that reactions in ILs yielded products of higher DP, when NMP as a co-solvent was used (sample **22**, DP_n 67 and sample **23**, DP_n 74). Co-solvents facilitate the conversion by decreasing the viscosity of the reaction medium and, hence, promoting a better miscibility. Moreover, reactions in IL

require less amounts of reagent and, hence, enable milder reaction conditions (molar ratio cellulose:NMP:TosCl of 1:2:2). Another advantage is that ILs can be recovered, purified, and reused in subsequent reactions. The recovery of NMP/LiCl is more complicated and higher amounts of both solvent and reagents are required (molar ratio cellulose:NMP:TosCl:Py of 1:168:5.5:5.5; NMP was used as solvent).

It is important to note that not only NMP but also other lactams (ε-caprolactam, *N*-methyl-ε-caprolactam, and *N*-methyl-2-piperidone) can be converted to the corresponding cellulose esters. Depending on the lactam used, ester moieties having different alkyl chain lengths (C_4 to C_6) can be attached to the cellulose backbone [91]. These reactions were previously performed by Dr. Susann Dorn during the course of her PhD studies [56, 91].

Structure characterization

The structure of the cellulose esters was elucidated by different spectroscopic techniques. FTIR spectra showed the typical features of cellulose-4-[*N*-methylammonium]butyrate chloride (Fig. 13).

The vibration corresponding to the N–CH_3 group at 2820–2760 cm^{-1} could not be clearly assigned in the FTIR spectra. Signals at 1733 cm^{-1} (νC=O) and 1260 cm^{-1} (νC–O–C) are characteristic for the ester moiety. The intensity of the νC=O vibration increases with increasing DS. The CH_2Cl vibration (present in the FTIR spectrum of sample **14**) was difficult to assign, but should be expected at wave numbers of approximately 1280 and 900 cm^{-1}.

Typical samples were investigated by means of NMR spectroscopy. The ^{13}C NMR spectra of cellulose backbone were according to the literature [4].

Spectra of the cellulose-4-[*N*-methylammonium]butyrate chlorides were well resolved and show all expected structural features (Fig. 14). In the case of a sample with low DS (DS of 0.31, **5**),

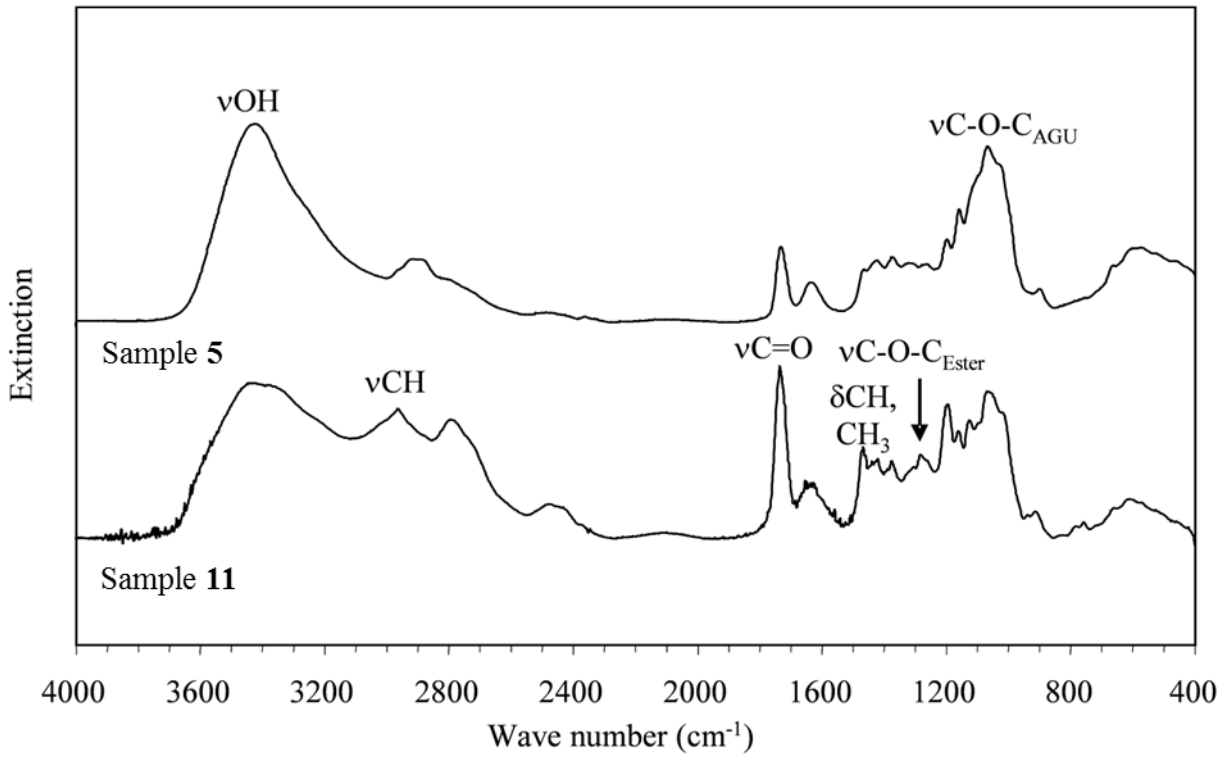


Fig. 13. FTIR spectra of cellulose-4-[*N*-methylammonium]butyrate chlorides, sample **5**, degree of substitution (DS) of 0.31 and sample **11**, DS of 1.01 (see Table 1).

two signals for position 6 were observed at 63.7 ppm (C-6$_{substituted}$) and at 60.9 ppm (C-6$_{OH}$) indicating esterification of position 6 as expected at this comparably low DS. Further signals were found at 173.3 ppm (C-7, C=O), 103.2 ppm (C-1, CH), 80.4 ppm (C-4, CH), 75.5–72.4 ppm (C-2, 3, 5, CH), 48.1 ppm (C-10, C-N), 32.9 ppm (C-11, CH_3), 31.1 (C-8, CH_2), and 21.3 ppm (C-9, CH_2).

There was no distinct indication for a functionalisation of the secondary positions. At a higher DS of 1.01 (sample **11**), the signal for the non-functionalized position 6 at 60.90 ppm is very weak with an increased intensity of the peak at 63.8 ppm (C-6$_{substituted}$). Therefore, it can be concluded that position 6 was almost completely functionalized.

However, splitting of the carbonyl signal at 172.3 and 171.5 ppm as well as splitting of the C-1 peak at 102.8 and 99.6 ppm indicated the esterification of position 2, to a certain extent.

Sample **14** (prepared at 60°C) contained a comparably low amount of nitrogen that corresponds

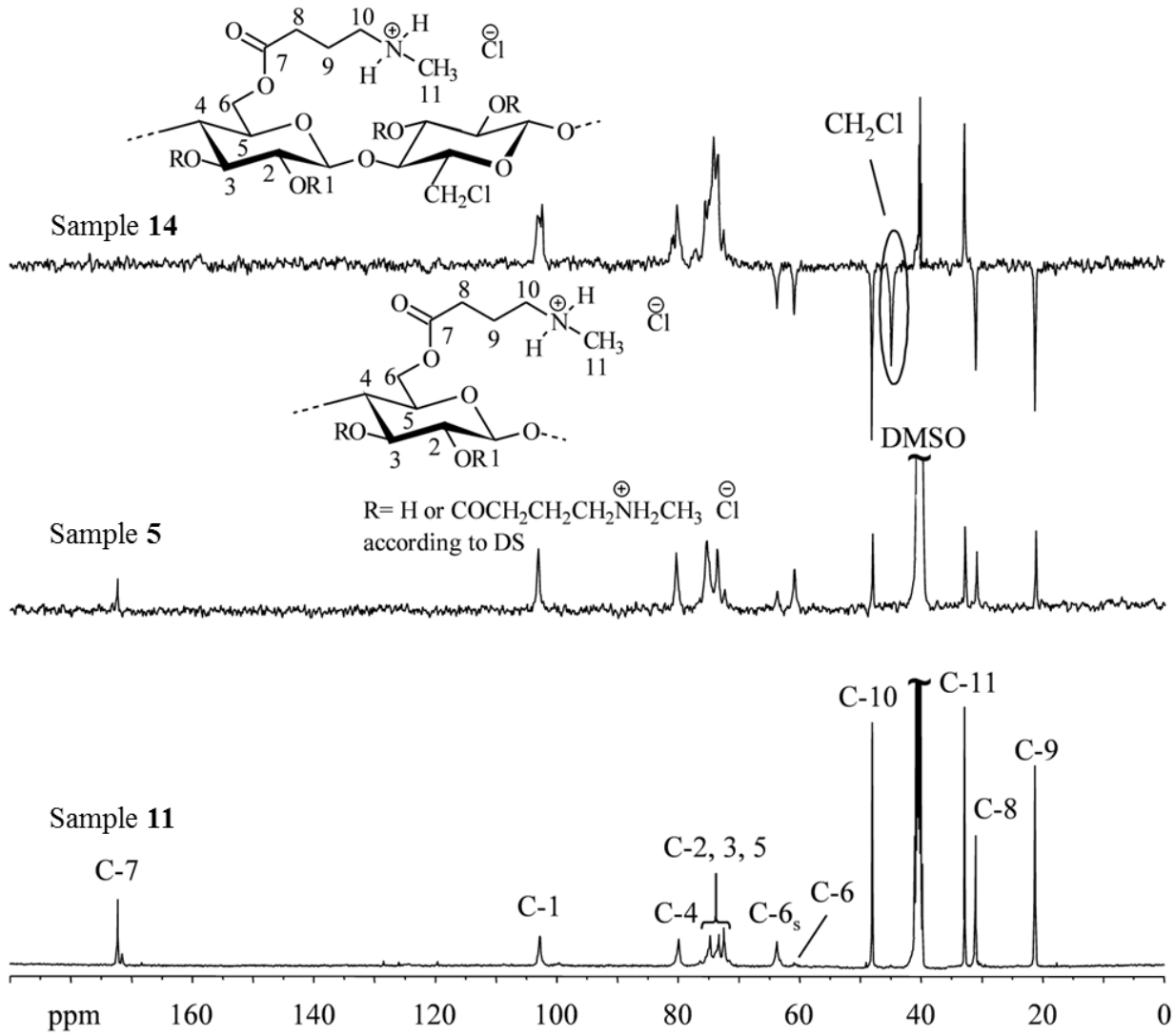


Fig. 14. ^{13}C NMR spectra of cellulose-4-[*N*-methylammonium]butyrate chlorides with different degree of substitution (DS, sample **14**, DS of 0.24, DEPT135 NMR spectrum; sample **5**, DS of 0.31; sample **11**, DS of 1.01) recorded in dimethylsulfoxide-d_6.

to a DS of 0.24 only, while a higher DS of up to 0.62 (sample **6**) could be realized at lower temperatures using the same molar ratio of AGU:NMP:TosCl. A new peak at 44.9 ppm appeared in the ^{13}C NMR spectrum of sample **14**. This was assigned as methylene group by DEPT135 NMR (Fig. 14). Thus, the formation of deoxychloro moieties at higher reaction temperatures could be demonstrated. The carbonyl carbon atom (C-7) did not appear in the DEPT 135 NMR spectrum due to the absence of protons bond to this atom. Position 6 was also partly esterified as

there were two signals at 63.7 ppm (esterified position 6). Thus, reaction temperatures > 40°C are not recommended due to the predominant occurrence of undesirable side-reactions. The resolution of the ^{1}H-NMR spectra was too low to acquire ^{1}H/^{1}H-COSY NMR spectra for the complete peak assignment. However, a rough peak assignment could be accomplished using the HSQC spectrum (Fig. 15).

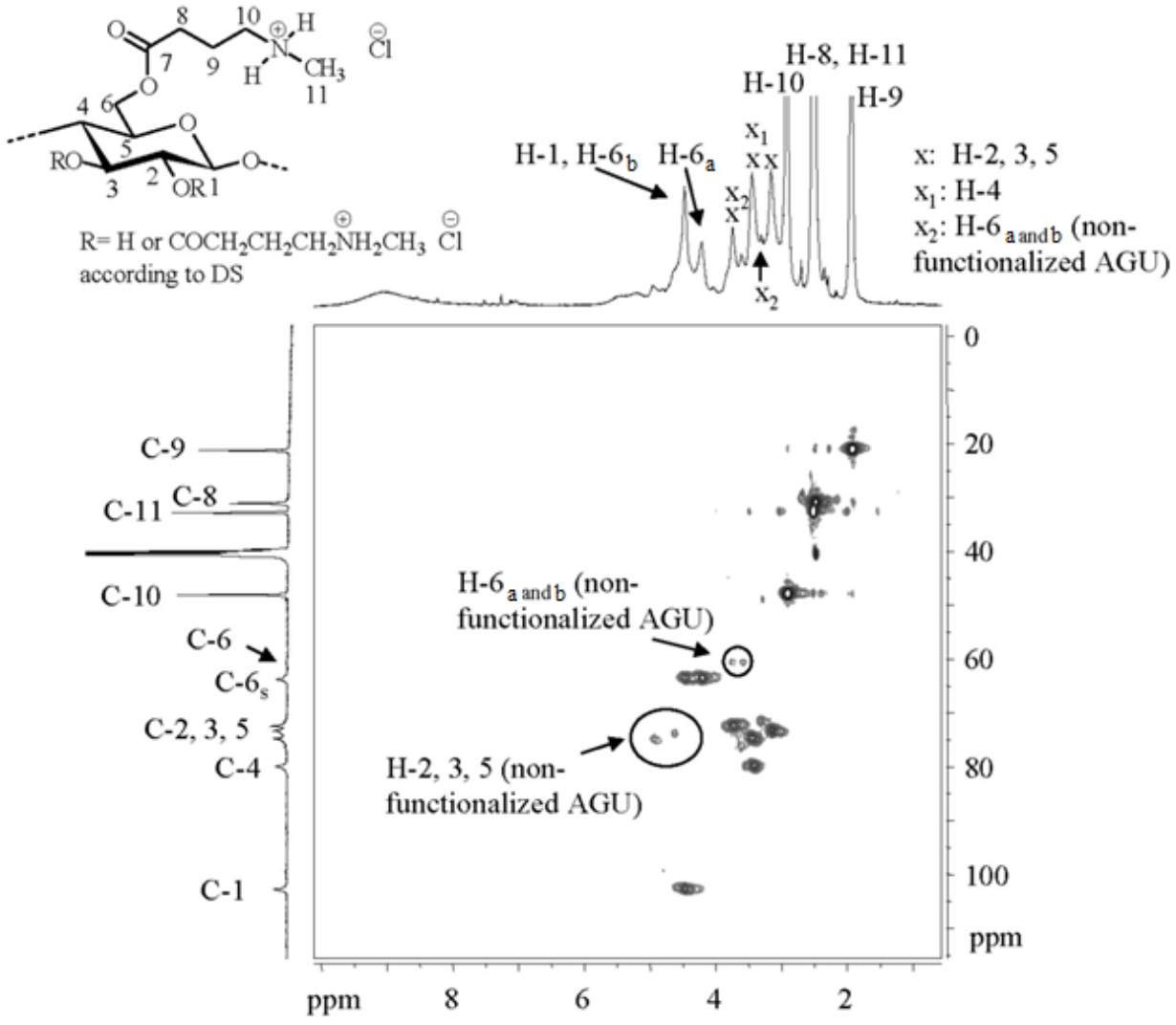


Fig. 15. HSQC NMR spectrum of cellulose-4-[*N*-methylammonium]butyrate chloride with degree of substitution (DS) of 1.01 (sample **11**) recorded in dimethylsulfoxide-d_6 (anhydroglucose unit, AGU).

For sample **11** (DS of 1.01), the following ^{1}H resonances were assigned: 4.48 ppm (H-1), 4.45, 4.29 ppm (H-6a, H-6b), 3.73, 3.45, 3.15 ppm (H-2, 3, 5), 3.45 ppm (H-4), 2.93 ppm (H-10), 2.53 ppm (H-11), 2.50 ppm (H-8), and 1.93 ppm (H-9). Moreover, two weak signals corresponding to the non-functionalized CH_2-group of position 6 appeared at 3.71 and 3.58 ppm. There were also two weak peaks that were detected which were tentatively assigned as position 2, 3, or 5 of a non-functionalized AGU.

The cellulose esters were isolated as their corresponding hydrochlorides and a complete presence of the salt form was assumed. However, the chlorine content of samples prepared at higher reaction temperature increased disproportionately to the nitrogen content. This is a clear hint for the occurrence of side reactions, that is, the formation of deoxychloro moieties in accord with NMR evidence previously mentioned. The samples were soluble in DMSO and water. Obviously, the hydrophobic nature of the alkyl chain is compensated by the cationic moiety at its terminus.

3.1.1.2. Synthesis and characterization of hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride

Hydroxyethyl cellulose (donated by Dow Wolff Cellulosics) was used as starting material in the synthesis of ammonium hydroxyethyl cellulose esters (Fig. 16).

In order to determine the molar degree of substitution of hydroxyethyl moieties (MS_{HEC}), treatment of the hydroxyethyl cellulose sample with acetyl chloride in DMAc/LiCl was carried out, yielding the peracetylated hydroxyalkyl ether without any hydroxyl groups (as confirmed by FTIR spectroscopy), which was well soluble in chloroform and DMSO. A MS of 3.66 was calculated on the basis of the corresponding ^{1}H NMR spectrum according Schaller & Heinze [92].

The ring-opening reaction of *N*-methyl-pyrrolidone was used for the modification of

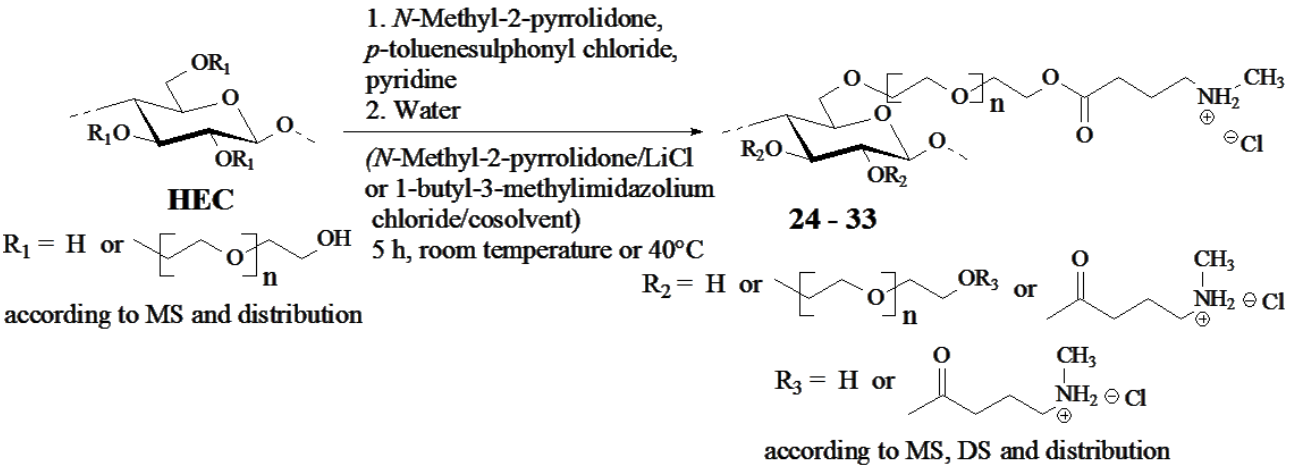


Fig. 16. Conversion of hydroxyethyl cellulose (HEC) with *N*-methyl-2-pyrrolidone in the presence of *p*-toluenesulphonyl chloride to obtain hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride. Degree of substitution, DS, and n is according to MS.

hydroxyethyl cellulose. The term 'DS' was used in this context comparably to reactions on unmodified cellulose since the total number of hydroxyl groups in HEC is also 3. The cellulose ether was dissolved in NMP and was allowed to react with TosCl and pyridine at different reaction conditions. The performed experiments are summarized in the Table 2. Samples in water- and DMSO-soluble were produced. In a first series of experiments, the polymer solution was allowed to react with 5 mol of TosCl and 5 mol of pyridine per mol of HEC yielding a hydroxyethyl cellulose ester with DS of 0.46 (sample **24**). The use of a lower molar ratio of 1:3:3 (HEC:TosCl:Py, data not shown) and reaction performed in the absence of pyridine (sample **25**), both under comparable reaction conditions, did not yield a hydroxyethyl cellulose ester.

By increasing the molar ratio to 1:10 (HEC:TosCl), a sample of comparable DS of 0.47 (sample **26**) was obtained without the use of pyridine. A sample of higher DS value of 0.75 (sample **28**) was yielded in the presence of pyridine at a comparable molar ratio and reaction time (5 h). Dissolution of HEC in NMP/LiCl does not yield products of higher DS. On the contrary, the DS was decreased (DS of 0.48, sample **29**) and a chlorine content of 9%, higher than the expected (5%), indicated the formation of deoxychloro moieties. A higher DS of 0.65 (sample **30**) with a

Table 2. Conditions for and results of the conversion of hydroxyethyl cellulose (MS of 3.66) dissolved in *N*-methyl-2-pyrrolidone/LiCl or 1-butyl-3-methylimidazolium chloride with *N*-methyl-2-pyrrolidone in the presence of *p*-toluenesulphonyl chloride.

Conditions						Results			
Solvent	Molar ratio[a]			Temp. (°C)[b]	Time (h)	Sample	Elemental analysis (%)		DS[c]
	NMP	TosCl	Py				N	Cl	
NMP	excess	5	5	RT	5	**24**	1.66	6.91	0.46
NMP	excess	5	-	RT	5	**25**	-	-	-
NMP	excess	10	-	RT	5	**26**	1.93	3.17	0.47
NMP	excess	10	-	10	5	**27**	-	-	-
NMP	excess	10	10	RT	5	**28**	2.47	4.83	0.75
NMP/LiCl	excess	10	10	RT	5	**29**	1.74	9.37	0.48
NMP/LiCl	excess	10	10	RT	16	**30**	2.22	14.11	0.65
NMP	excess	15	15	RT	5	**31**	3.00	12.87	0.98
NMP	excess	30	30	10	5	**32**	2.39	2.86	0.72
BMICl/NMP	10	4	-	40	5	**33**	1.09	12.23	0.28

[a] Molar ratio of modified anhydroglucose unit:*N*-methyl-2-pyrrolidone:*p*-toluenesulphonyl chloride: pyridine; [b] room temperature (RT); [c] degree of substitution (DS) calculated from the nitrogen content.

high chlorine content of 14% was achieved by prolonging the reaction time from 5 to 16 h at both comparable molar ratio (1:10:10, HEC:TosCl:Py) and reaction temperature. The formation of deoxychloro moieties might be caused by the presence of LiCl (high content of Cl^-) in the reaction mixture. Thus, a sample of increased DS of 0.98 (sample **31**) was obtained in absence of LiCl after 5 h at room temperature, but also with a high chlorine content of 13%.

The formation of deoxychloro moieties is facilitated when cellulose is reacted in the presence of TosCl at high reaction temperatures (80-100°C) via *in situ* formation of tosyl cellulose [93]. Due to the large number of primary hydroxyl groups compared to cellulose, side-reactions like formation of 6-deoxy-6-chloro moieties become more dominant in case of HEC (Fig.17). No

conversion was observed in the isolated sample **27**, using a lower molar ratio (1:10, HEC:TosCl) at 10°C. However, a sample with DS of 0.72 was obtained at higher molar ratio at the same temperature (molar ratio of 1:30:30, HEC:TosCl:Py, sample **32**) without an excess of chlorine content after a comparable reaction time. With increasing molar ratio, the chemical equilibrium was shifted to the formation of hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride, and low temperatures (10°C) prevented the side-reaction occurrence. Sulphur content from tosylate anions also was determined by elemental analysis in some samples. The anions were easily exchanged by Cl^- using an ion exchange resin.

The conversion of HEC was also studied in BMIMCl solution containing NMP as co-solvent. A sample with DS of 0.28 (sample **33**) was produced but with a high chlorine content of 12%. Even with the addition of a co-solvent and a reaction temperature of 40°C, the reaction medium was very viscous which hindered proper mixing of the reagents and, hence, product formation.

DP_n of 508 and DP_w of 1096 (PDI = 2.16) were determined for HEC (starting material) dissolved in DMAc/LiCl by SEC. However, the DP of the products could not be determined using the same equipment. Analysis of the polymeric cationic amine salt of hydroxyethyl cellulose by SEC is tricky due to the ionic interactions of the ammonium groups with the residual anionic end-groups of the column packing materials. This interaction results in abnormal size exclusion

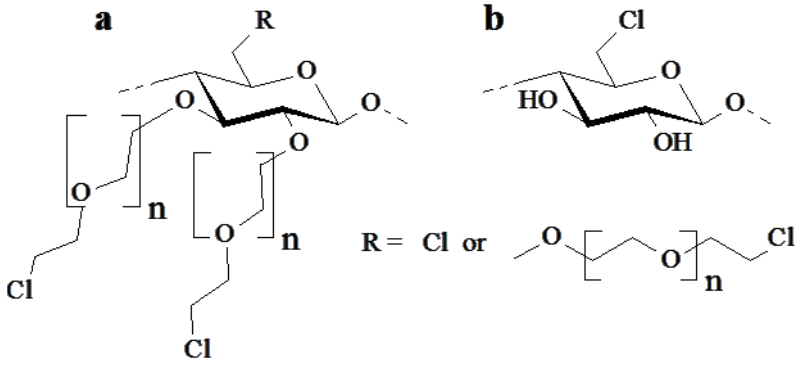


Fig. 17. Possibilities of substitution of primary hydroxyl groups by deoxychloro moieties in hydroxyethyl cellulose (a) and in cellulose (b).

separation, such as poor chromatographic peak shape, low recovery and increased retention times, thus rendering erroneous determination of molecular weights and molecular weight distributions of the sample [94].

Structure characterization

The structure of the hydroxyethyl cellulose esters had been studied by different spectroscopic techniques. The FTIR spectra showed the typical features of hydroxyethyl cellulose and hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride (Fig. 18).

The strong and broad OH signal at 3438 cm^{-1} indicated the presence of hydroxyl groups in both cellulose derivatives. The signals around 2922 cm^{-1} are attributed to the aliphatic alkyl (CH_2, CH_3) vibrations. The carbonyl moiety is assigned at 1736 cm^{-1} in the spectrum of the

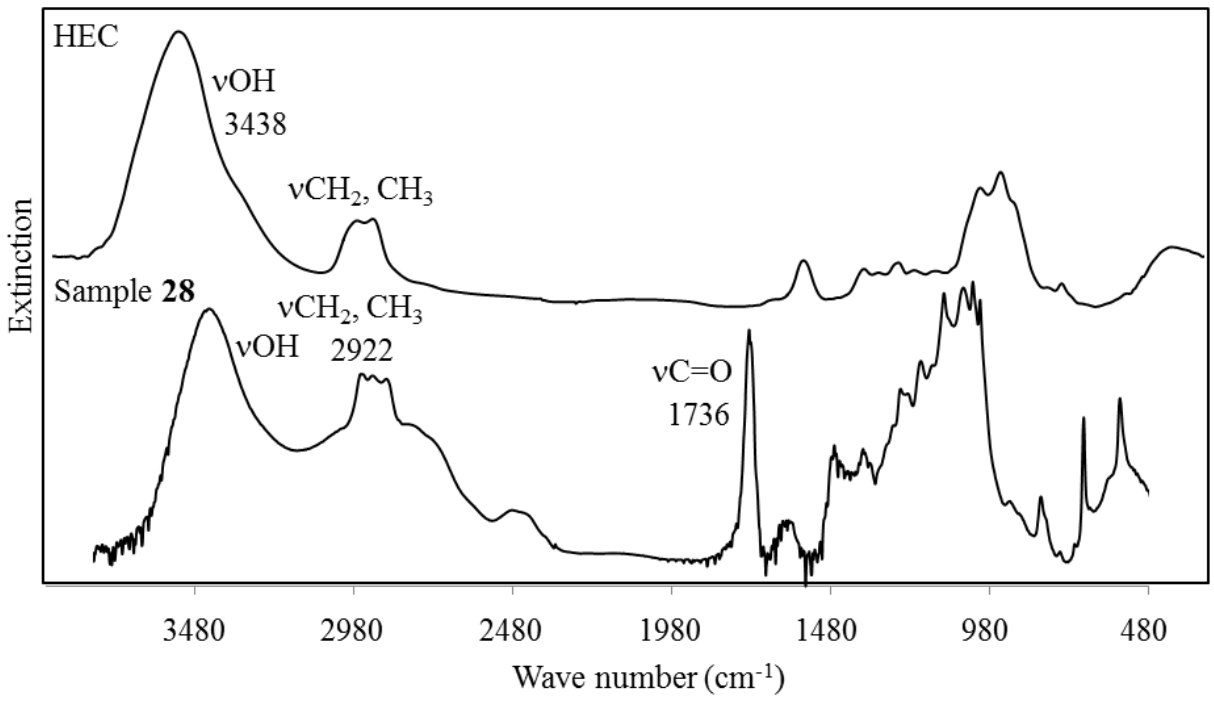


Fig. 18. FTIR spectra of hydroxyethyl cellulose (HEC) and hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride, sample **28**, degree of substitution of 0.75 (see Table 2).

hydroxyethyl cellulose ester. The vibration corresponding to the N–CH_3 group at 2820 – 2760 cm^{-1} could not be clearly assigned.

Typical samples were investigated by means of NMR spectroscopy. The samples were very viscous, which interfered with the acquisition of clear signals for the hydroxyethyl cellulose backbone. ^{13}C NMR spectra of the hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chlorides showed all the expected structural features (Fig. 19).

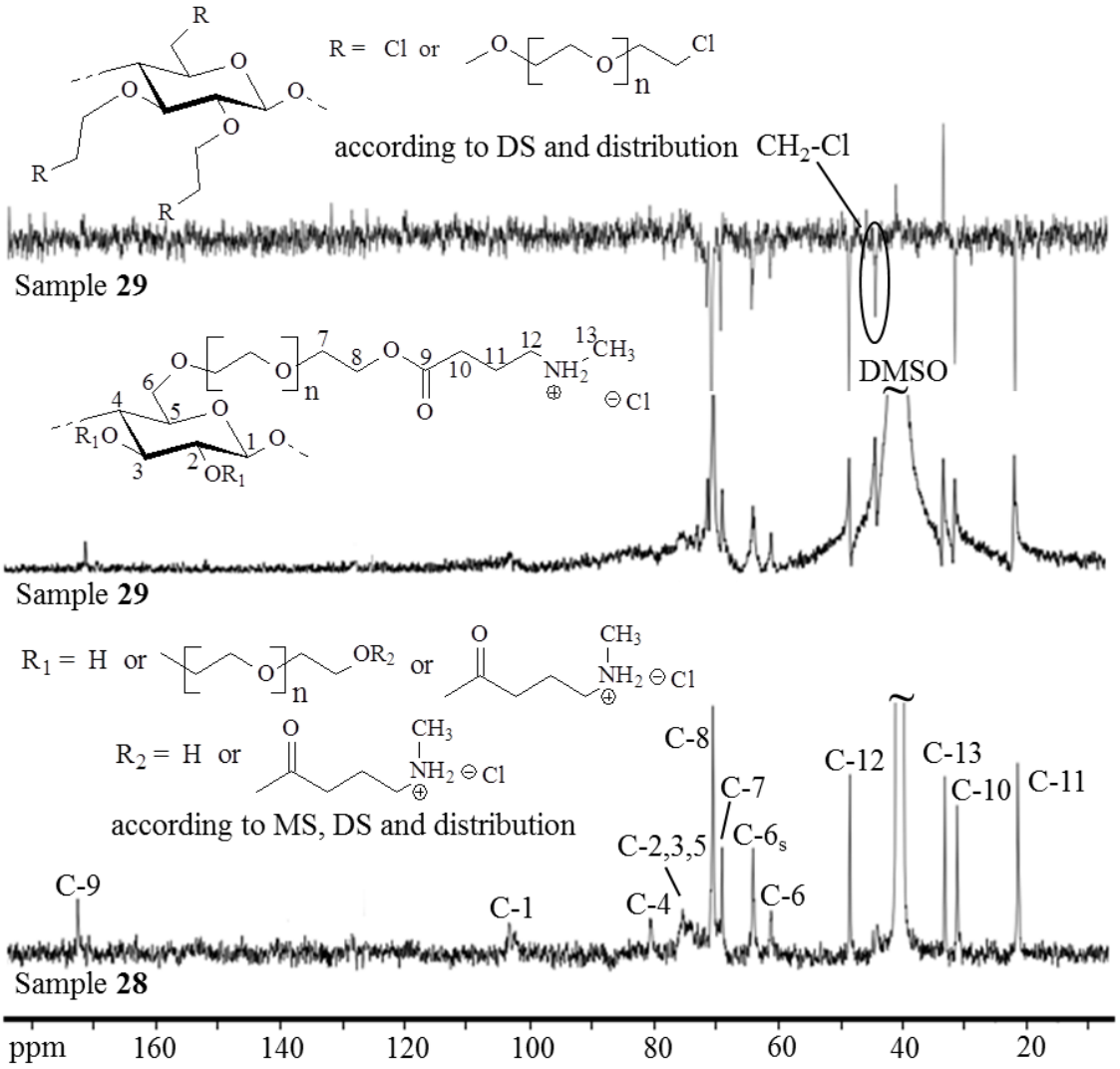


Fig. 19. ^{13}C NMR spectra of hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chlorides with different degree of substitution (DS) (sample **29**, DS of 0.48, DEPT135 NMR spectrum; sample **28**, DS of 0.75) recorded in dimethylsulfoxide-d_6

Sample **28** (DS of 0.75) presented two signals for position 6 at 63.8 ppm (C-$6_{substituted}$) and at 60.9 ppm (C-6_{OH}) indicating unreacted hydroxyl groups at this position.

Further signals were found at 172.4 ppm (C-9, C=O), 103.1 ppm (C-1, CH), 80.4 ppm (C-4, CH), 75.2–71.7 ppm (C-2, 3, 5, CH), 70.3 ppm (C-8, CH_2), 68.8 ppm (C-7, CH_2), 48.3 ppm (C-12, C-N), 33.0 ppm (C-13, CH_3), 31.0 ppm (C-10, CH_2) and 21.3 ppm (C-11, CH_2). Due to the poor resolution, substitution at C-2 and C-3 was difficult to identify.

The ^{13}C NMR spectrum of a sample with high chlorine content (sample **29**) obtained after 5 h at room temperature and in the presence of LiCl presented a more pronounced peak at 43.9 ppm (Fig. 19). In DEPT135 NMR spectroscopy, a negative signal appeared indicating a methylene group, which was assigned as CH_2Cl moiety [93]. Thus, the formation of deoxychloro moieties could be proven. Reactions performed at room temperatures in the presence of LiCl applying an excess of reagents (molar ratio AGU:TosCl:Py higher than 1:5:5) are not recommended for the conversion of HEC due to the predominant occurrence of side-reactions. A rough peak assignment could be accomplished using the HSQC spectrum (Fig. 20).

For sample **28** (DS of 0.75), the following ^1H resonances were assigned: 4.17 ppm (H-6_s in substituted carbon), 3.56 ppm (H-6), 3.22 ppm (H-2, 3, 4, 5, 6, 7, 8), 2.91 ppm (H-12), 2.51 ppm (H-13), 2.31 ppm (H-10), and 1.92 ppm (H-11). Assignment in the H-1 was not possible due to the low resolution. The hydroxyethyl cellulose esters were isolated as their corresponding hydrochlorides. For the calculation of the DS from the elemental analysis data, a complete presence of the salt form was assumed. However, the chlorine content of samples prepared at room temperature in the presence of LiCl and of an excess of reagents (molar ratio AGU:TosCl:Py higher than 1:5:5) increased disproportionately to the nitrogen content. This is a clear hint for the occurrence of side reactions, that is, the formation of deoxychloro moieties in accordance with NMR data previously mentioned. The samples were soluble in DMSO and water.

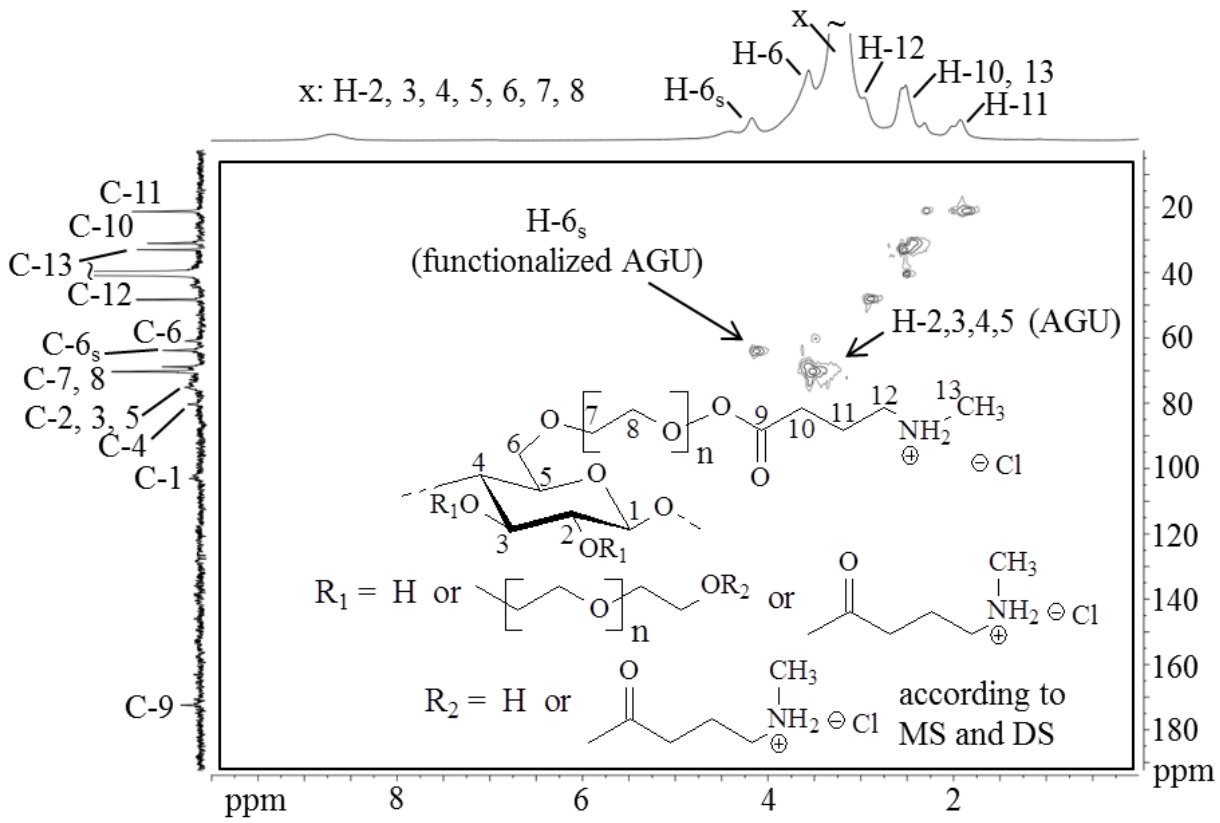


Fig. 20. HSQC NMR spectrum of hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride with degree of substitution (DS) of 0.75 (sample **28**) recorded in dimethylsulfoxide-d_6 (anhydroglucose unit, AGU). MS, molar degree of substitution, DS, degree of substitution, and s, substituted.

3.1.1.3. Synthesis and characterization of dextran-4-[*N*-methylammonium]butyrate chloride

Dextran was allowed to react with *N*-methyl-2-pyrrolidone in presence of *p*-toluenesulphonyl chloride and pyridine (Fig. 21). The reaction conditions and the results are summarized in the Table 3.

In a first series of experiments, 2 g of dextran dissolved in NMP/LiCl was allowed to react with 5.5 mol of TosCl and 5.5 mol of pyridine per mol of anhydroglucose unit (AGU). Dextran-4-[*N*-methylammonium]butyrate chloride with DS of 0.30 (sample **34**) was obtained after a reaction

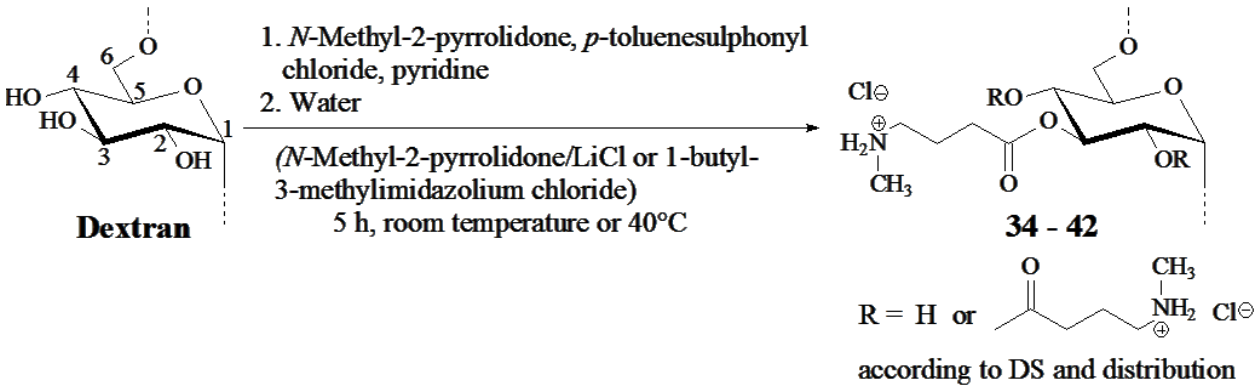


Fig. 21. Conversion of dextran with *N*-methyl-2-pyrrolidone in the presence of *p*-toluenesulphonyl chloride to obtain dextran-4-[*N*-methylammonium]butyrate chloride. DS, degree of substitution.

time of 2 h. Increasing the reaction time to 5 h resulted in a product with a slight higher DS of 0.39 (sample **36**). Under the same reaction conditions, sample **38** (DS of 0.25) was obtained without addition of pyridine. A product of higher DS (sample **39**, DS of 0.52) was generated by increasing the reaction time to 16 h. Reactions performed at 60°C using cellulose as a starting material yielded side-product containing deoxychloro moieties at position 6 (Table 1, sample **14**). However, the use of dextran allows the increase of temperature during the conversion with NMP/TosCl since nucleophilic substitution reaction is more difficult at secondary hydroxyl functions (Fig. 21, positions 2, 3 and 4 of dextran AGU).

Nevertheless, nucleophilic substitution must be taken into account mainly because it contains branches with primary OH moieties at their end groups [84, 95]. Thus, a sample with a similar DS of 0.53 (sample **41**, Table 3) was obtained at 60°C without an excess of chlorine (calculated chlorine content: 8.05%, found: 8.03%). Dextran-4-[*N*-methylammonium]butyrate chlorides of lower DS were obtained with increasing molar ratio of 1:11:11 (AGU:TosCl:Py, results not shown). These experiments demonstrated that the molar ratio of 1:5.5:5.5 (AGU:TosCl:Py) gives the highest DS values.

The samples were DMSO- and water-soluble. Samples of higher DS (sample **35**, **37** and **40**)

Table 3. Conditions for and results of the conversion of dextran (DP_n = 236, DP_w= 465, PDI = 1.97) dissolved in *N*-methyl-2-pyrrolidone/LiCl or 1-butyl-3-methylimidazolium chloride in the presence of *p*-toluenesulphonyl chloride.

Conditions						Results							
Solvent	Molar ratio[a]			Temp. (°C)[b]	Time (h)	Sample	Elemental analysis (%)			DS[c]	DP_n[d]	DP_w	PDI
	NMP	TosCl	Py				N	Cl	S				
NMP/LiCl	excess	5.5	5.5	RT	2	**34**	1.72	3.45	0.65	0.30	115	274	2.38
NMP/LiCl	excess	5.5	5.5	RT	2	**35**[e]	3.20	7.76	-	0.54	140	377	2.68
NMP/LiCl	excess	5.5	5.5	RT	5	**36**	2.71	5.52	1.18	0.39	116	267	2.30
NMP/LiCl	excess	5.5	5.5	RT	5	**37**[e]	3.50	8.34	-	0.61	159	366	2.31
NMP/LiCl	excess	5.5	-	RT	5	**38**	1.78	3.89	-	0.25	118	282	2.39
NMP/LiCl	excess	5.5	5.5	RT	16	**39**	3.14	6.38	1.36	0.52	143	305	2.13
NMP/LiCl	excess	5.5	5.5	RT	16	**40**[e]	3.88	9.00	-	0.72	127	343	2.71
NMP/LiCl	excess	5.5	5.5	60	16	**41**	3.18	8.03	1.01	0.53	114	348	3.04
BMIMCl	2	2	-	RT	5	**42**	0.91	2.16	-	0.12	140	256	1.83

[a] Molar ratio anhydroglucose unit:*N*-methyl-2-pyrrolidone:*p*-toluenesulphonyl chloride: pyridine; [b] room temperature (RT); [c] degree of substitution (DS) calculated from the nitrogen content; [d] degree of polymerization (DP) calculated from $\overline{M}_n$ and $\overline{M}_w$; [e] upscale reaction performed with 4 g of starting material.

were afforded using 4 g of dextran after 2 h, 5 h and 16 h at room temperature.

A reaction was performed using an ionic liquid (BMIMCl) as an alternative solvent to dissolve dextran (sample **42**). BMIMCl is a promising solvent for dextran: a DMSO and water-soluble sample with a low DS of 0.12 was obtained after 5 h, even using a lower molar ratio of 1:2:2 (AGU:NMP:TosCl). At comparable reaction time, a product with higher DS of 0.39 (sample **36**) was obtained with dextran dissolved in NMP/LiCl, however a higher molar ratio of 1:5.5:5.5 (AGU:NMP:TosCl) was used.

The samples presented similar degrees of polymerization in the range of $114 \leq DP_n \leq 159$ and of $256 \leq DP_w \leq 377$. Starting dextran had a DP_n of 236 and DP_w of 465; this means that the samples were degraded to a certain extent during their modification.

The composition of the samples was determined by elemental analysis. Their chlorine content was in agreement to their nitrogen content. However, some samples (**34**, **36**, **39** and **41**) contained sulphur. Several purification steps including dialysis and ion exchange were applied in any dextran sample in order to remove the tosylate counterion of the ammonium group.

Structure characterization

The structure of the amino dextran esters was elucidated by different spectroscopic techniques. FTIR spectrum showed the typical features of dextran-4-[*N*-methylammonium]butyrate chloride (sample **40**, Fig. 22). The strong and broad OH signal at 3338 cm^{-1} indicated the presence of hydroxyl groups due to the DS of 0.72. The signal around 2928 cm^{-1} is attributed to the aliphatic alkyl (CH_2, CH_3) vibrations as well. The vibration corresponding to the N–CH_3 group at 2820–2760 cm^{-1} could not be clearly assigned in the FTIR spectra. The characteristic peak of ester moiety is evidenced at 1735 cm^{-1} (νC=O). The peak at 1645 cm^{-1} is due to the first overtone of O–H bending. SO_2 vibrations were difficult to assign in the spectrum of samples containing

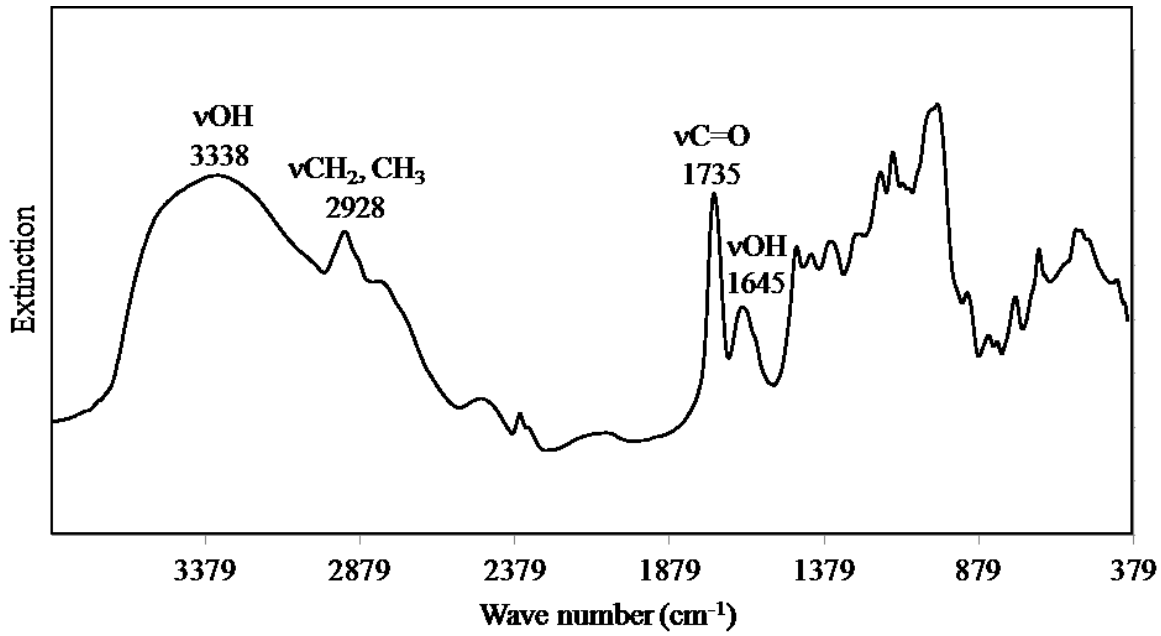


Fig. 22. FTIR spectrum of dextran-4-[*N*-methylammonium]butyrate chloride (sample **40**, degree of substitution of 0.71).

sulfur (**34**, **36**, **39** and **41**, Table 3, spectra not shown). However, such signals should be expected at wave numbers of approximately 1359 cm^{-1} and 1175 cm^{-1} [93].

The structure of the samples was investigated by means of NMR spectroscopy (Fig. 23). An exact peak assignment was not possible by two-dimensional NMR spectroscopy due to insufficient resolution. Thus, the substitution at position 2, 3 or 4 in the AGU was difficult to discern. However, the structure was clearly shown by ^{13}C-NMR spectroscopy, and all expected structural features were observable. In the case of a sample with DS of 0.12 (Fig. 23, sample **42**), signals for the ester moiety in the range from 20 to 50 ppm (aliphatic region) and 172 ppm (carbonyl signal) and for the AGU (60 – 100 ppm) were detected. Substitution at position 2 in the sample of higher DS (**39**, DS of 0.52) was observed by the split of the signal C-1 (98.8 ppm) at 95.91 ppm (C-1').

Samples containing sulfur (**34**, **36**, **39** and **41**, Table 3) showed two small signals at 128.5 ppm and 126.0 ppm by ^{13}C NMR (Fig. 23, sample **39**). These signals were assigned to the chemical shifts of the aromatic carbon atoms of tosylate anion.

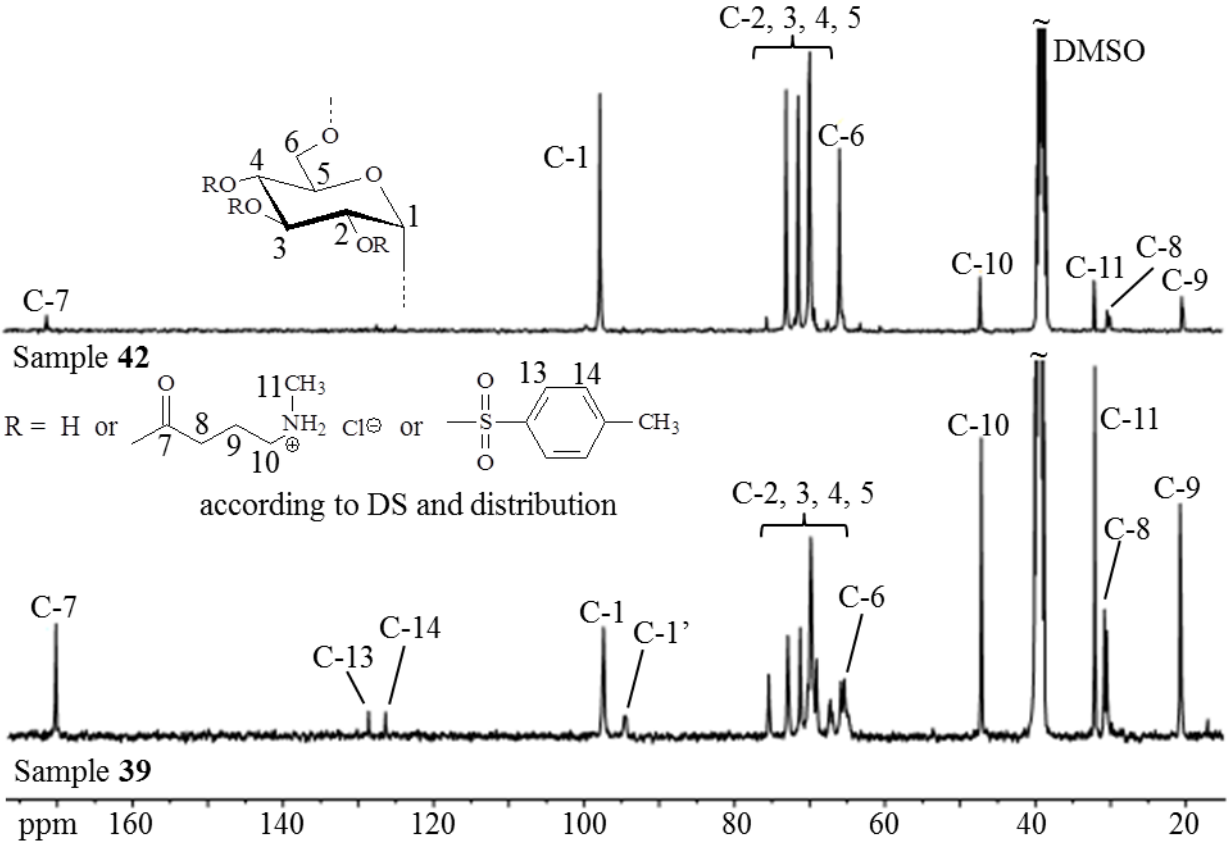


Fig. 23. ^{13}C-NMR spectra of dextran-4-[*N*-methylammonium]butyrate chlorides (sample **42**, DS of 0.12, and sample **39**, DS of 0.52). Measurements were carried out in DMSO-d_6. DS, degree of substitution.

The influence of ion exchange treatment of the samples was studied by ^{1}H NMR spectroscopy. Covalently bond tosyl moieties lead to broad peaks at 7.81 ppm and 7.49 ppm, whereas tosylate anions acting as counterions of the ammonium group were observed at 7.53 ppm and 7.13 ppm (sample **39**, Fig. 24). Formation of deoxychloro unit was excluded since no further signal in the region around 42 ppm was observed. Tosylation of dextran requires a long reaction time (24 h) and is carried out at a low reaction temperature (around 8°C, for instance) [95]. Tosyl dextran was formed to a low extent as well. Covalently bond tosyl moieties are hard to remove; it might be possible under reductive conditions with $LiAlH_4$ [96].

In order to confirm the nature of the tosyl groups linked to dextran chain, an aq. solution of

sample **39** (0.02%, w/v, after purification with ion exchange resin) was analysed by UV-vis spectroscopy. Methyl 6-O-tosyl-α-D-glucopyranoside (MTG) was used as standard (λ_{max} = 227 nm).

The covalent bond of the tosyl moiety was proven as the dextran ester containing sulfur and methyl 6-O-tosyl-α-D-glucopyranoside had the same λ_{max}. Thus, a calibration curve was performed using MTG, and a low amount of 1.81% (w/w) of tosyl groups was determined for this sample (Fig. 25). This result was comparable to its sulfur content from elemental analysis (1.36%, Table 3).

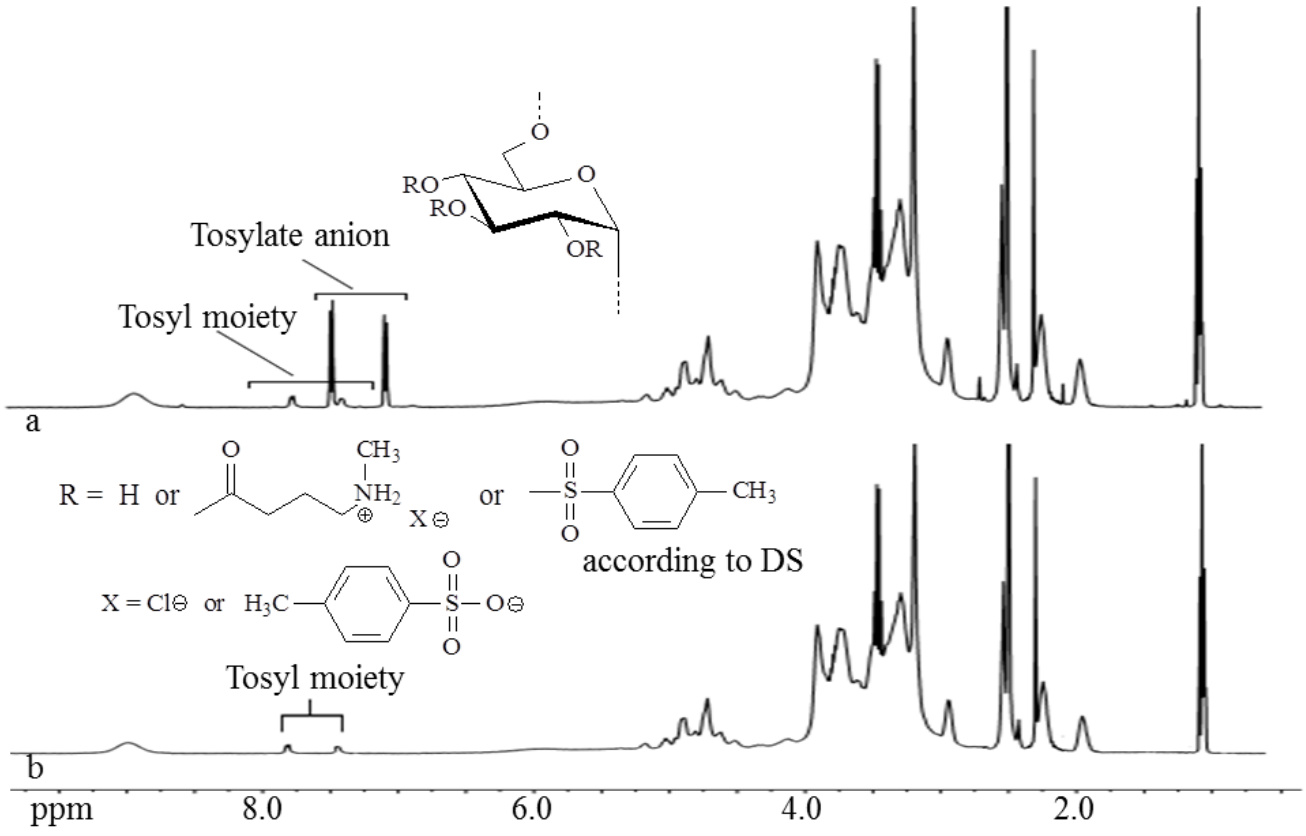


Fig. 24. [1]H-NMR spectra of dextran-4-[*N*-methylammonium]butyrate chloride (sample **39**, DS of 0.52) before (a) and after (b) purification of sample with ionic exchanger. Measurements were carried out in DMSO-d_6.

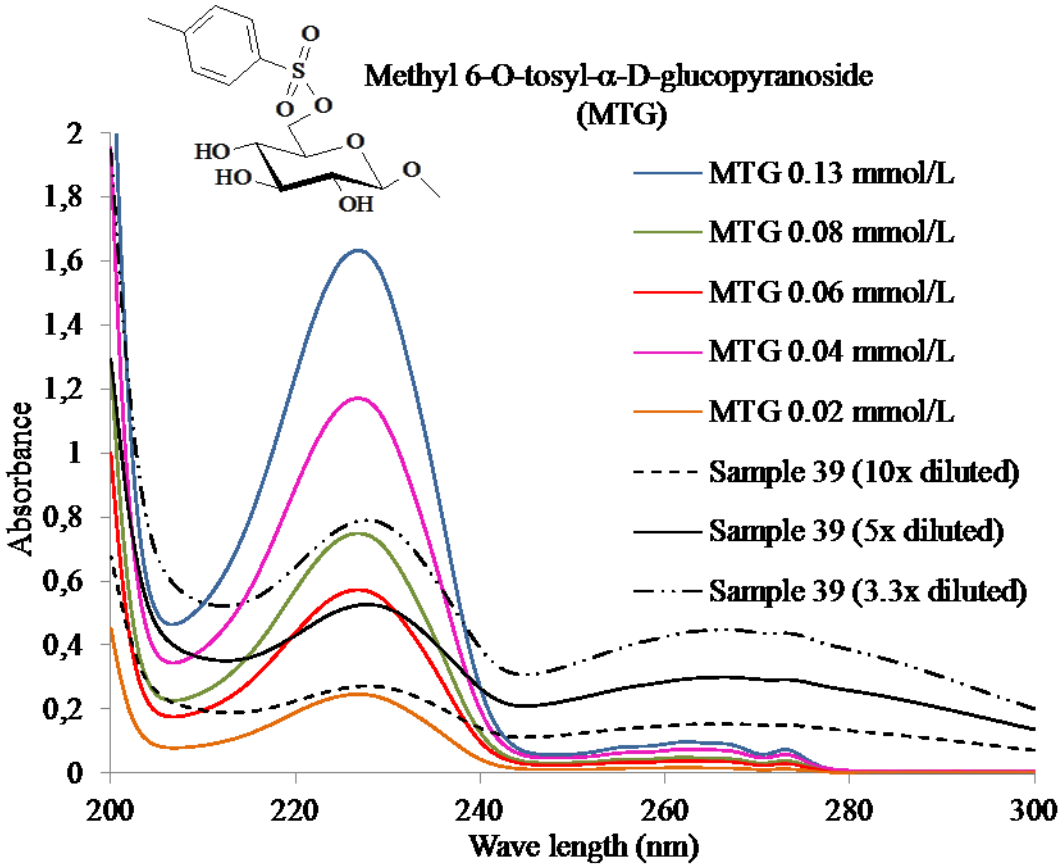


Fig. 25. UV-vis absorbance spectra of sample **39** in aq. solution (0.02%, w/v, 3.3, 5 and 10 times diluted) and of aq. methyl 6-O-tosyl-α-D-glucopyranoside (MTG, λ_{max} = 227 nm) measured at different concentrations.

3.1.2. Esterification of cellulose with (3-carboxypropyl)trimethyl ammonium chloride activated by *N,N'*-carbonyl diimidazole

Novel cellulose-4-[*N,N,N*-trimethylammonium]butyrate chlorides were homogeneously synthesized via activation of (3-carboxypropyl)trimethylammonium chloride (CPTAC) with *N,N'*-carbonyldiimidazole (CDI, Fig. 26).

Cellulose-4-[*N,N,N*-trimethylammonium]butyrate chlorides were prepared by a two step synthesis using the separately synthesized (3-carboxypropyl)trimethyl imidazolide to investigate optimal reaction conditions. (3-Carboxypropyl)trimethyl ammonium chloride (CPTAC) was

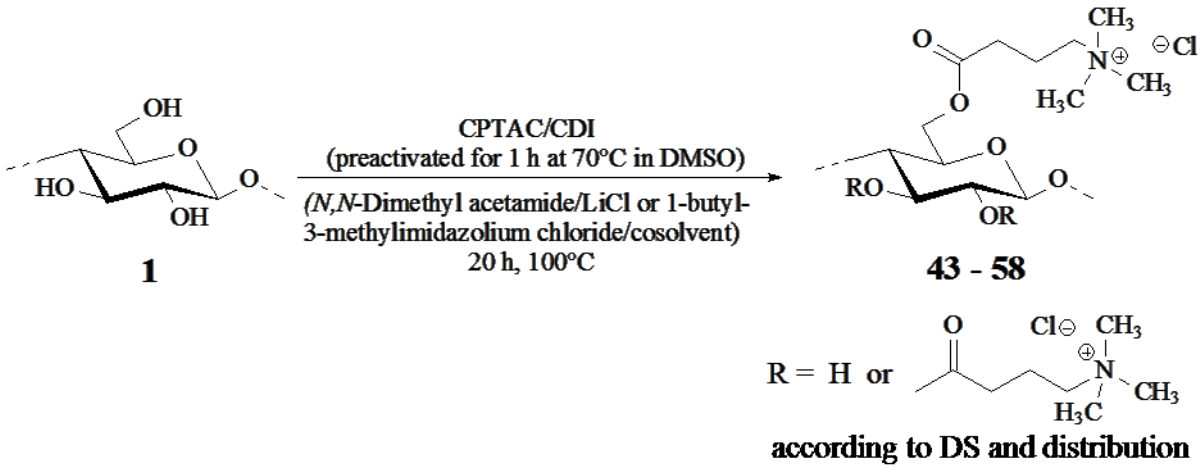


Fig. 26. Conversion of cellulose **1** with (3-carboxypropyl)trimethyl ammonium chloride (CPTAC), previously activated with *N,N'*-carbonyl diimidazole (CDI), to obtain cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride. Degree of substitution, DS.

allowed to react with *N,N'*-carbonyl diimidazole (CDI), resulting in (3-carboxypropyl)trimethyl imidazolide (first step A_1, Fig. 27), which was converted with the biopolymer to cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride (second step A_2, Fig. 27). *In situ* activation of carboxylic acids with CDI can also be carried out in a one-pot reaction. However, alcoholic hydroxyl groups may also react yielding undesired cross-linking by carbonate formation in the case of a polyol (single step B, Fig. 27) [39, 97].

In the first series of experiments, cellulose **1** dissolved in DMAc/LiCl was allowed to react with 1 mol of (3-carboxypropyl)trimethylammonium chloride (CPTAC), previously activated with 1 mol of CDI per mol of AGU yielding a insoluble cellulose ester with DS of 0.12 (Table 4, sample **43**). Water- and DMSO-soluble samples were obtained by increasing reaction time: a DS of 0.36 was realized after 20 h at 70°C (sample **44**). Increasing molar ratio to 1:2:2, 1:3:3 and 1:5:5 (AGU:CPTAC:CDI) afforded samples with DS of 0.21 (sample **46**), 0.31 (sample **51**) and 0.38 (sample **52**), respectively, after 20 h at 70°C. These experiments showed that no higher DS values were achieved using an excess of reagents.

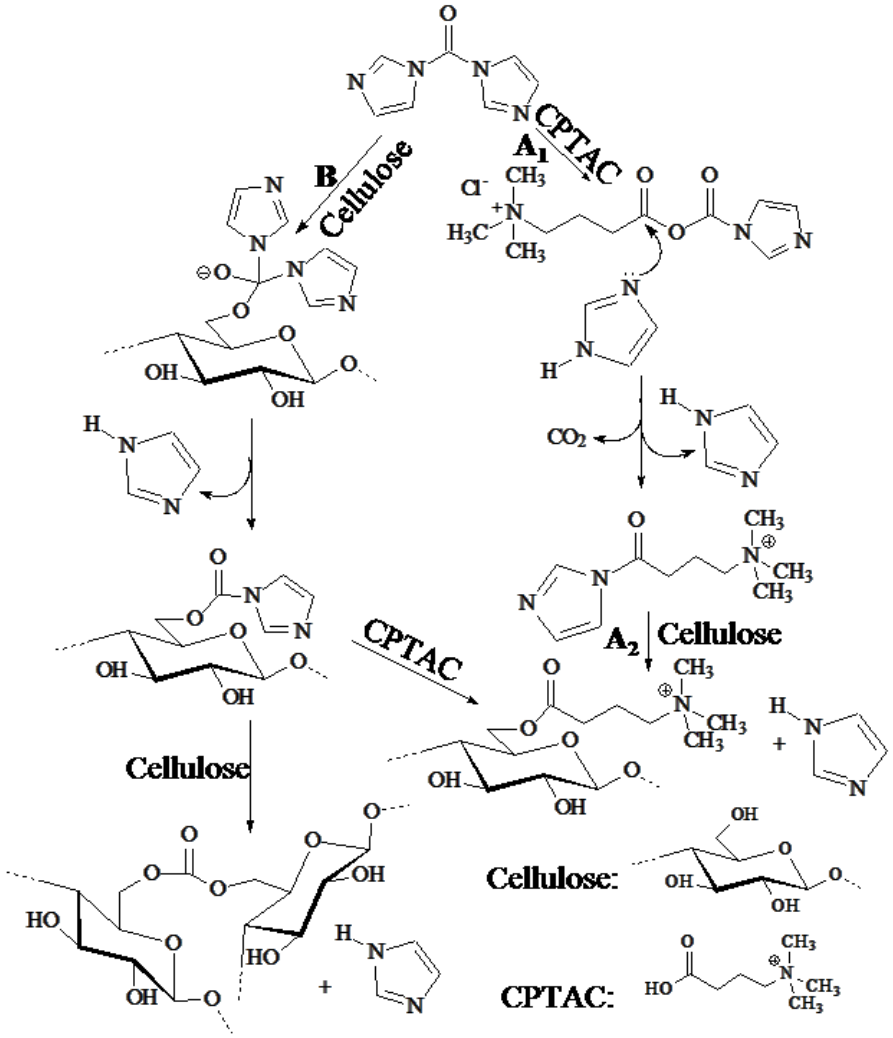


Fig. 27. Reaction pathway leading to esterification in two steps (A_1 and A_2) or cross-linking (B) if the cellulose is treated directly with *N,N'*-carbonyldiimidazole. CPTAC means (3-carboxypropyl)trimethylammonium chloride. Adapted from reference [98].

Cellulose **1** was dissolved according to the literature in TBAF/DMSO and a reaction using this different reaction medium was performed [99]. A sample with a slightly higher DS of 0.39 (sample **48**) was obtained at same reaction conditions compared to a sample synthesized in DMAc/LiCl (sample **46**, DS of 0.21). On the other hand, it was found that increasing the reaction temperature to 100°C yielded samples with higher DS of 0.49 (sample **45**, molar ratio of 1:1:1, AGU:CPTAC:CDI) and DS of 0.75 after 20 h (sample **49**, molar ratio of 1:2:2, AGU:CPTAC:CDI). Further increase of reaction time to 48 h did not yield product with a higher

Table 4. Conditions for and results of the conversion of cellulose **1** (DP_{cuen} of 156, DP_n 99, DP_w 339, PDI 3.42) dissolved in *N,N*-dimethylacetamide/LiCl or 1-butyl-3-methylimidazolium chloride in the presence of (3-carboxypropyl)trimethylammonium chloride activated with *N,N'*-carbonyldiimidazole in dimethylsulfoxide.

Conditions					Results						
Cellulose solvent	Molar ratio[a]		Temp. (°C)	Time (h)	Sample	Elemental analysis (%)		DS[c]	DP_n[d]	DP_w	PDI
	CPTAC	CDI				N	Cl				
DMAc/LiCl	1	1	70	5	**43**[b]	0.91	3.09	0.12	n.d.	n.d.	n.d.
DMAc/LiCl	1	1	70	20	**44**	2.28	5.61	0.36	63	299	4.77
DMAc/LiCl	1	1	100	20	**45**	2.82	7.16	0.49	31	231	7.42
DMAc/LiCl	2	2	70	20	**46**	1.52	4.37	0.21	45	218	4.88
DMAc/LiCl	2	2	70	20	**47**[e]	2.48	6.24	0.40	124	569	4.60
TBAF/DMSO	2	2	70	20	**48**	2.41	4.94	0.39	26	245	9.62
DMAc/LiCl	2	2	100	20	**49**	3.69	8.80	0.75	18	72	3.86
DMAc/LiCl	2	2	100	48	**50**	3.47	8.93	0.68	22	129	5.89
DMAc/LiCl	3	3	70	20	**51**	2.02	5.11	0.31	41	201	4.85
DMAc/LiCl	5	5	70	20	**52**	2.14	3.44	0.38	16	53	3.33
BMIMCl	1	1	80	5	**53**[b]	0.83	2.63	0.11	n.d.	n.d.	n.d.
BMIMCl	1	1	80	16	**54**[b]	1.05	2.85	0.14	n.d.	n.d.	n.d.
BMIMCl/DMSO	1	1	80	5	**55**	1.21	3.85	0.16	64	358	5.59

Table 4. (cont.)

Conditions					Results						
Cellulose solvent	Molar ratio[a]		Temp. (°C)	Time (h)	Sample	Elemental analysis (%)		DS[c]	DP_n[d]	DP_w	PDI
	CPTAC	CDI				N	Cl				
BMIMCl	2	2	80	5	**56**	1.27	3.15	0.17	88	483	5.46
BMIMCl	2	2	80	16	**57**	3.22	8.43	0.60	50	393	7.87
BMIMCl	2	2	100	5	**58**	3.64	10.03	0.73	35	357	10.24

[a] Molar ratio anhydroglucose unit: (3-carboxypropyl)trimethylammonium chloride: *N,N'*-carbonyldiimidazole; [b] sample water- and DMSO-insoluble, not determined (n.d.); [c] degree of substitution (DS) calculated from the nitrogen content; [d] degree of polymerization (DP) calculated from $\overline{M}_n$ and $\overline{M}_w$; [e] reaction performed with cellulose **3** (DP_{cuen} = 1795, DP_n 1702, DP_w 15430, PDI 9.06).

DS. On the contrary, the DS was decreased to 0.68 (sample **50**). It must be pointed out that also cotton linters (cellulose **3**) could be converted to water-soluble cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride with a reasonable DS of 0.40 (sample **47**) using a molar ratio of 1:2:2 (AGU:CPTAC:CDI) after 20 h at 70°C.

In a further series of experiments, the ionic liquid 1-butyl-3-methylimidazolium chloride (BMIMCl) as a single component solvent for cellulose and for the imidazolide was used. It was reported to be the most efficient reaction medium for esterifications using carboxylic acids and CDI, and this solvent was also applied in the reactions with (3-carboxypropyl)trimethylammonium chloride [63]. The conversion of cellulose **1** with 1 mol of CPTAC, in the presence of 1 mol of CDI, at 80°C in BMIMCl afforded water-insoluble products with a DS of 0.11 (after 5 h, sample **53**) and a DS of 0.14 (after 16 h, sample **54**). DMSO as a co-solvent was added to the reaction in order to decrease the viscosity and to evaluate its effect on the degree of substitution of the product. However, the use of co-solvent did not yield a sample with higher DS (DS of 0.16, sample **55**). Increasing the molar ratio to 1:2:2 (AGU:CPTAC:CDI) afforded cellulose-4-[*N,N,N*-trimethylammonium]butyrate chlorides with a DS of 0.17 (after 5 h, sample **56**) and 0.60 (after 16 h, sample **57**) at 80°C. Reactions in ionic liquids were more efficient than reactions performed in DMAc/LiCl since a product with DS of 0.73 was obtained in a short reaction time of 5 h (sample **58**) at 100°C and comparable molar ratio. As noted above, ionic liquids can be recovered, purified and reused in further reactions. The use of CDI as activating agent did not provide products with a higher degree of polymerization (Table 4) comparing to cellulose esters generated by ring-opening of NMP (Table 1). Polymer degradation was observed in all reactions mainly due to the use of high reaction temperature (70° - 100°C). This is in accordance with the literature: a decrease of molecular weight by 40% was observed during the esterification of bacterial cellulose with 3,6-dioxaheptanoic acid and/or 3,6,9-trioxadecanoic acid *in situ* activated by CDI at 90°C [63].

Structure characterization

The structure of the cationic cellulose esters was elucidated by a variety of different spectroscopic techniques. FTIR spectra showed the typical features of cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride (Fig. 28). The broad signal at 3333 cm^{-1} was assigned to OH stretching vibration indicating a partial substitution of hydroxyl groups of anhydroglucose unit. The signals at 2906 cm^{-1} and 1157 cm^{-1} are due to the C–H stretching and C–O stretching vibrations, respectively. The peak at 1645 cm^{-1} is due to the first overtone of O–H bending due to adsorbed water [100]. The presence of additional signals at 2127 cm^{-1} and 1420 cm^{-1} were assigned to the C–N stretching vibration of ammonium groups [101]. This signal could be clearly identified in FTIR-spectra of cellulose-4-[*N,N,N*-trimethylammonium]butyrate chlorides although a signal of lower intensity in the spectra of 4-[*N*-methylammonium]butyrate chlorides

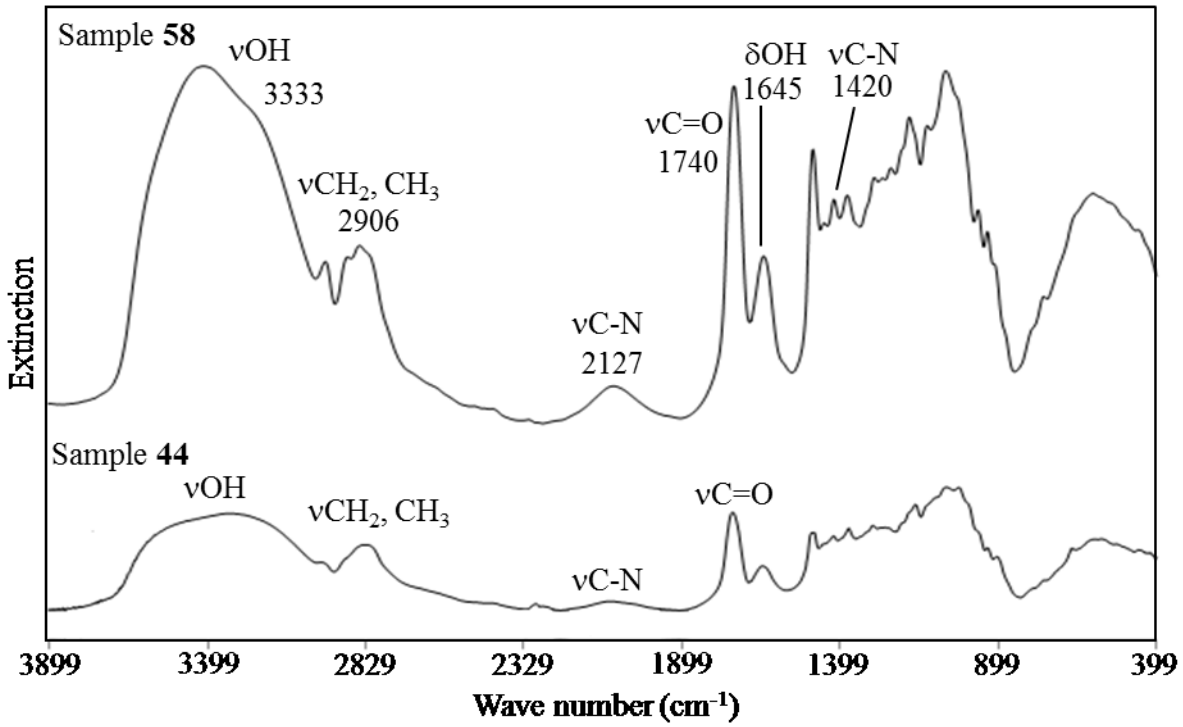


Fig. 28. FTIR spectra of cellulose-4-[*N,N,N*-trimethylammonium]butyrate chlorides, sample **44**, degree of substitution (DS) of 0.36 and sample **58**, DS of 0.73.

from cellulose and dextran appeared. The carbonyl moiety (νC=O) is assigned at 1740 cm^{-1}. The intensity of the νC=O vibration increases with increasing DS.

Typical samples were also investigated by means of NMR spectroscopy. The ^{13}C-NMR spectra of the cellulose-4-[*N,N,N*-trimethylammonium]butyrate chlorides (Fig. 29) were well resolved

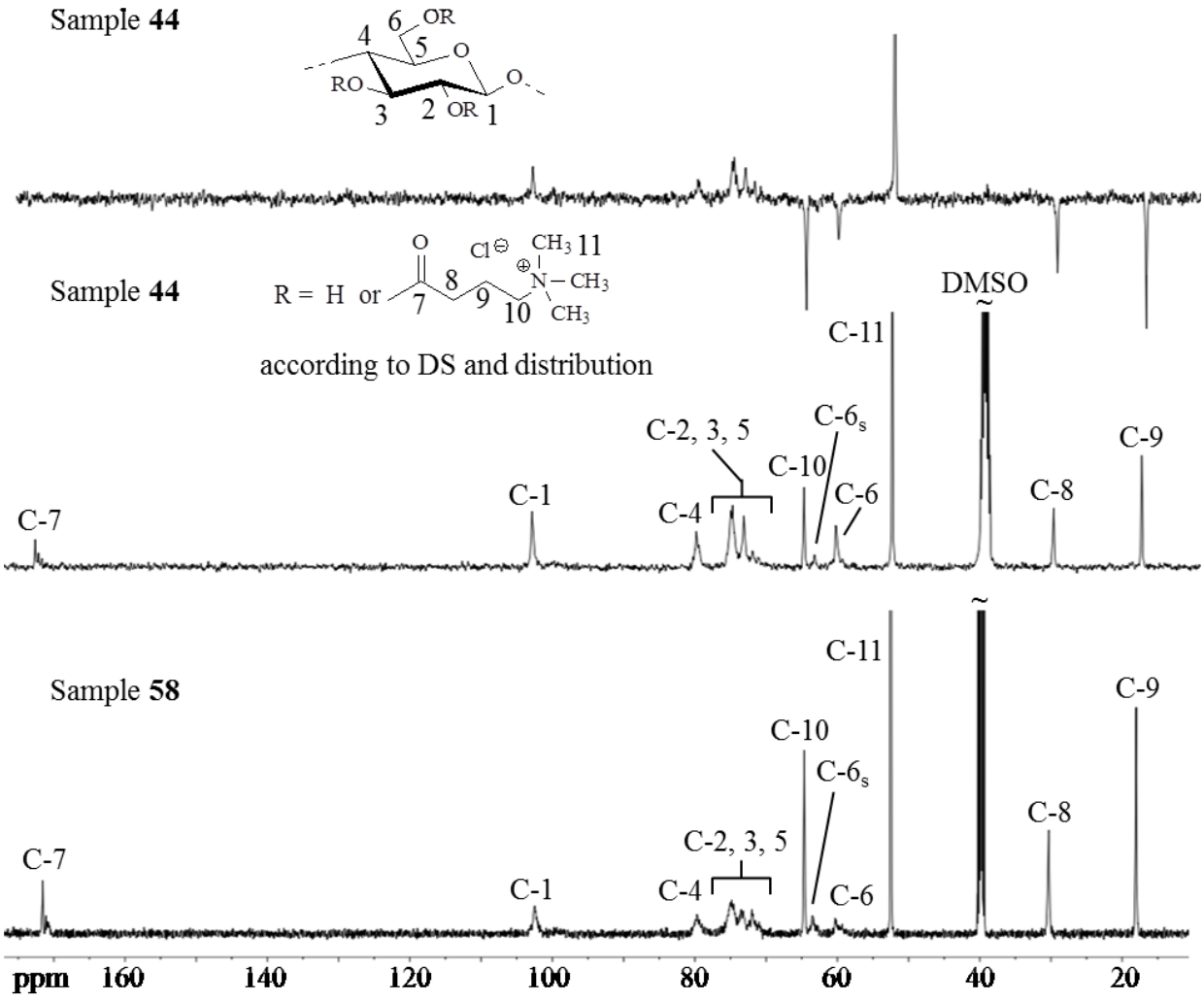


Fig. 29. ^{13}C-NMR spectra of cellulose-4-[*N,N,N*-trimethylammonium]butyrate chlorides with different degree of substitution (DS): sample **44**, DS of 0.36, DEPT 135 NMR spectrum, and sample **58**, DS of 0.73, recorded in dimethylsulfoxide-d_6. Index s means substituted by a functionalisation of the adjacent position.

and show all expected structural features. In the case of a sample with a low DS (DS of 0.36, **44**), two signals for position 6 were observed at 63.9 ppm (C-$6_{substituted}$, CH_2) and at 61.0 ppm (C-6_{OH}, CH_2) indicating esterification of position 6 as expected at this comparatively low DS. Further signals were found at 172.1 ppm (C-7, C=O), 103.1 ppm (C-1, CH), 80.3 ppm (C-4, CH), 75.5 – 72.5 ppm (C-2, 3, 5, CH), 65.4 ppm (C-10, CH_2), 53.1 ppm (C-11, CH_3), 30.7 ppm (C-8, CH_2), and 18.5 ppm (C-9, CH_2). At a higher DS of 0.73 (sample **58**), the signal for the non-functionalized position 6 at 61.0 ppm is weak with slightly increased intensity of the peak at 63.9 ppm (C-$6_{substituted}$). There was no distinct indication for a functionalisation of the secondary positions in the NMR spectra of cellulose-4-[*N,N,N*-trimethylammonium]butyrate chlorides. Therefore, it can be concluded that a preferred substitution at position 6 occurred. The peaks of the methyl and methylene groups were confirmed by DEPT 135 NMR (Fig. 29, sample **44**). A carbonyl carbon atom (C-7) is not visible in the DEPT135 NMR spectrum due to the absence of hydrogen bonded to this atom. Occurrence of side-reactions were not evidenced by the NMR analysis of cellulose-4-[*N,N,N*-trimethylammonium]butyrate chlorides.

A rough peak assignment was accomplished using the HSQC spectrum (Fig. 30). For sample **44** (DS of 0.36), the following ^{1}H resonances were assigned: 4.48 and 4.24 ppm (H-6_α, H-6_β), 4.37 ppm (H-1), 3.39 ppm (H-4, 5, 10), 3.08 ppm (H-2, 3), 3.05 ppm (H-11), 2.48 ppm (H-8), and 1.97 ppm (H-9). Moreover, two weak signals corresponding to the non-functionalized CH_2-group of position 6 appeared at 3.76 and 3.63 ppm.

The cellulose esters were isolated as their corresponding hydrochlorides. For the calculation of the DS from the elemental analysis data, a complete presence of the salt form was assumed. As a typical sample, the elemental composition of sample **44** (2.28% of nitrogen and 5.61% of chlorine, Table 4) agreed very well with the calculated values (2.28% of nitrogen and 5.77% of chlorine).

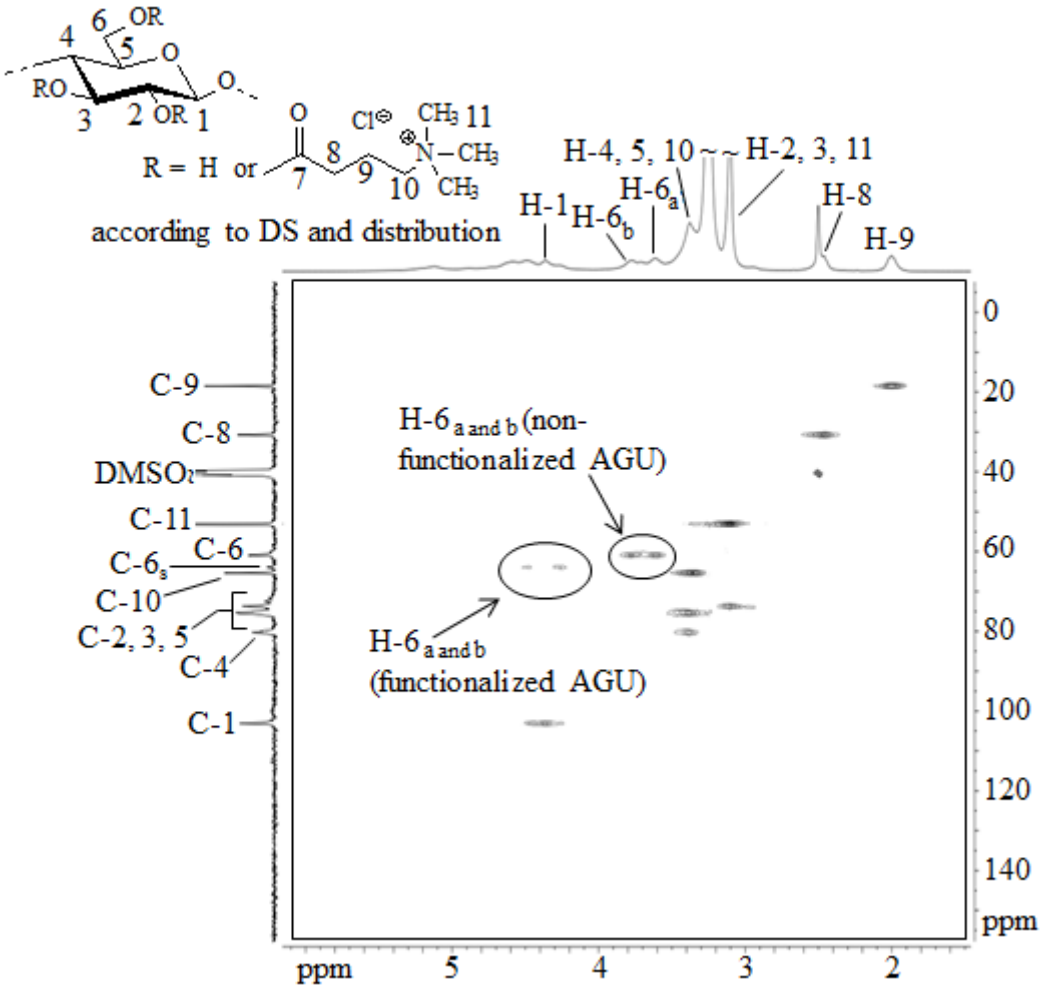


Fig. 30. HSQC NMR spectrum of cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride with degree of substitution (DS) of 0.36 (sample **44**) recorded in dimethylsulfoxide-d_6 (anhydroglucose unit, AGU).

3.1.3. Comparison of esterification methods

Two different esterification methods were applied for the modification of cellulose in this work. Both reactions yielded water-soluble products with a low degree of substitution and polymer degradation occurred in any case. Esterifications using TosCl as activating agent were performed under mild reaction conditions (16 h, at room temperature) but presented undesirable side-reactions with the formation of deoxychloro- and tosyl moieties. The conversion using CDI was

strongly reaction temperature-dependent (20 h, at 100°C). However, side-reactions and residual imidazole were not detected. As a suggestion for further research, 4-[methylammonium]butyric acid chloride (MABA) activated with CDI is recommended to be utilized for the esterification of cellulose in order to yield cellulose-4-[*N*-methylammonium]butyrate chlorides.

3.2. Charging behavior and stability of cellulose-4-[*N*-methylammonium]butyrate chloride and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride

Water-soluble ammonium group-containing cellulose esters are typically characterized as polyelectrolytes since they can be positively charged in aqueous solution. Cellulose-4-[*N*-methylammonium]butyrate chloride is protonated and positively charged in the acidic pH range (Fig. 31). On the other hand, cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride (Fig. 26) is a cellulose ester containing a tetraalkylammonium ion and it remains positively charged independently of the pH of the solution.

It is essential to analyse the cationic character of amino moieties due to the fact that its charges show an attractive electrostatic interaction with negatively charged polyelectrolytes and surfaces. The protonation behaviour and the stability of cellulose-4-[*N*-methylammonium]butyrate chloride and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride at different pH values

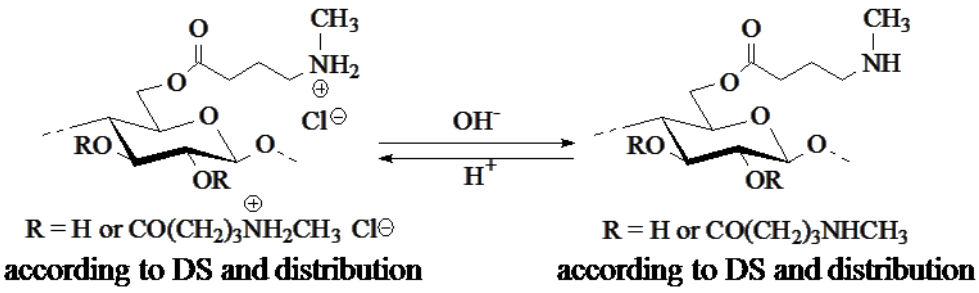


Fig. 31. Protonation equilibrium of cellulose-4-[*N*-methylammonium]butyrate chloride at acidic and alkaline solutions. Degree of substitution, DS.

were studied. Esters linkages may be sensitive to hydrolysis in aqueous media, particularly under alkaline conditions. Therefore, the stability of the cellulose derivatives was investigated at different storage times (up to 28 days) by potentiometric and polyelectrolyte titrations as well as by IR- and Raman spectroscopy.

3.2.1. Charging behaviour studied by polyelectrolyte titration

In order to determine the amount of positive charges, polyelectrolyte titrations of sample solutions were carried out in a Mettler Toledo DL 53 titrator using polyvinylsulfonic acid (PVS, 10 mM) as titrant and *O*-toluidine blue (0.1 mM) as indicator. The sample solution is turned from blue (excess of cationic charges) to rose (excess of anionic titrant) during the titration in presence of the indicator. Polycations are titrated with polyanions (PVS) assuming a 1:1 binding stoichiometry of vinylsulfonate to ammonium groups. Detailed explanation about the polyelectrolyte titration method is found in the literature [102]. The concentration of protonated amino groups was determined from the equivalent volume of PVS solution added, detected at the steepest rise in the curve of absorbance vs. volume of PVS (Fig. 32). The polyelectrolyte titration curves for cellulose-4-[*N*-methylammonium]butyrate chlorides (sample **5** and **11**, Table 1) and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride (sample **44**, Table 4) samples in the range of the pH values from 2.02 to 9.21 are shown in Fig. 32. The titrations were performed comparatively quickly (typically within 7 min), and the amounts of positive charges determined are summarized in Table 5.

The positive charges of sample **5** (DS of 0.31) are decreased by 67% when subjected to alkaline media, that is from 1.98 mmol/g (at pH = 2.02) to 0.65 mmol/g (at pH 8.70). For sample **11** (DS of 1.01), the decrease is larger: at pH 2.04, 3.94 mmol/g of positive charges is measured, while at pH 8.89, only 0.81 mmol/g of protonated amino groups was determined (79%). As expected, the charge decrease is more pronounced for the sample **2** which has a higher DS. The decrease in

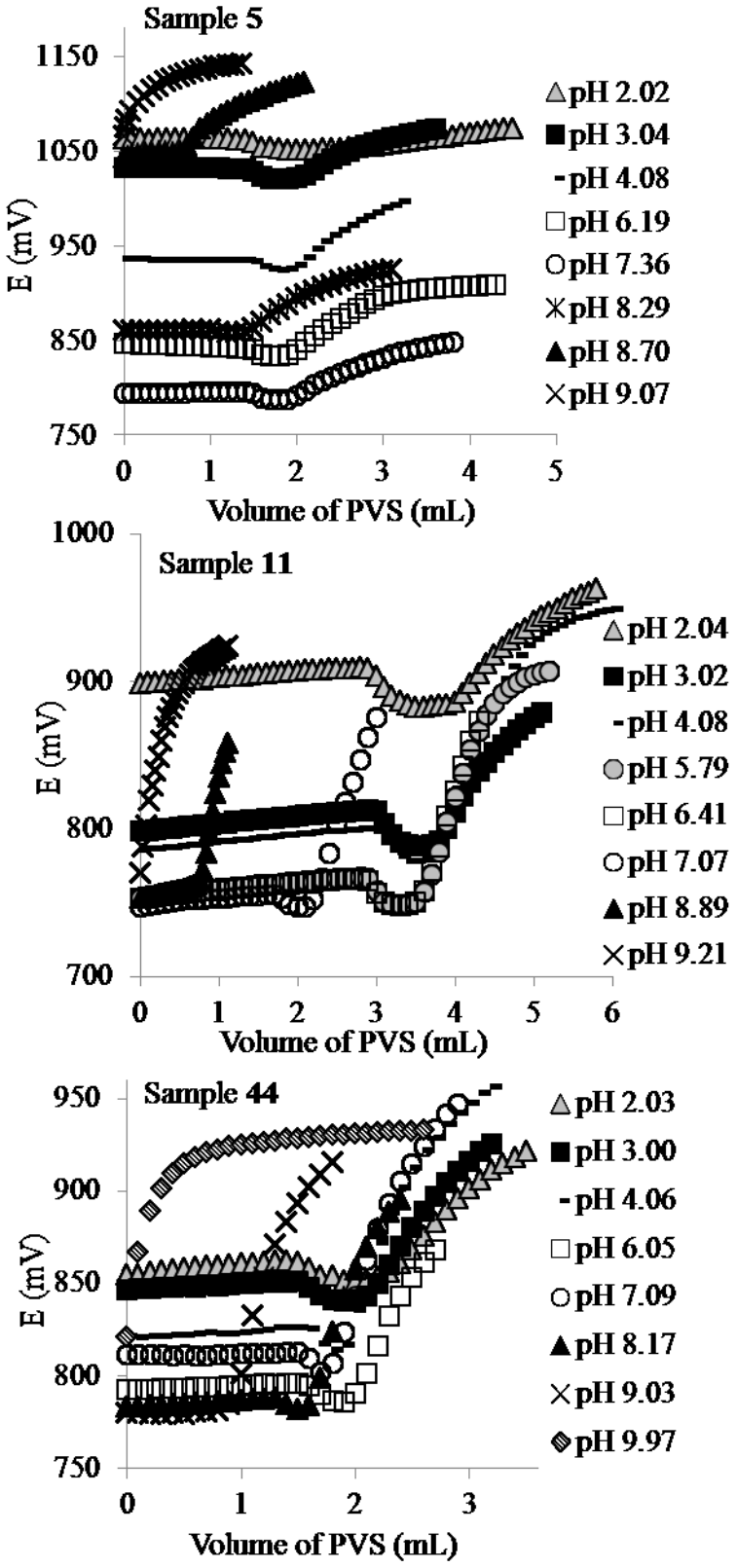


Fig. 32. Polyelectrolyte titration curves of aqueous cellulose-4-[*N*-methylammonium]butyrate chlorides (sample **5**, degree of substitution, DS, of 0.31, and sample **11**, DS of 1.01), and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride (sample **44**, DS of 0.36), 0.1%, w/v,

titrated with aq. polyvinylsulfonic acid (PVS, 10 mM) at different pH values. E = 1000 mV means 100% transmittance at λ = 660 nm.

positive charges with increasing pH is attributed to the deprotonation of ammonium moieties (Fig. 31) of such samples. At pH > 9, no positive charge was detected by this method, thus it indicated that both samples were totally deprotonated. However, the positive charges of sample **44** (permanent cation), also are decreased by 62% when subjected to alkaline media, that is, from 2.19 mmol/g (at pH = 2.03) to 0.83 mmol/g (at pH 9.03).

Table 5. Amount of positive charges of cellulose-4-[*N*-methylammonium]butyrate chlorides (sample **5** and **11**) and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride (sample **44**) measured by polyelectrolyte titration at different pH values.

Sample **5** (DS[a] of 0.31)		Sample **11** (DS[a] of 1.01)		Sample **44** (DS[a] of 0.36)	
pH value[b]	Amount of positive charges (mmol/g)[c]	pH value[b]	Amount of positive charges (mmol/g)[c]	pH value[b]	Amount of positive charges (mmol/g)[c]
2.02	1.98	2.04	3.94	2.03	2.19
3.04	1.98	3.02	3.86	3.00	1.99
4.08	2.01	4.08	3.74	4.06	1.89
6.19	1.92	5.79	3.64	6.05	1.89
7.36	1.92	6.41	3.60	7.09	1.76
8.29	1.44	7.07	2.22	8.17	1.63
8.70	0.65	8.1	1.37	9.03	0.83
9.07	n.d.[d]	8.51	0.94	9.97	n.d.[d]
		8.89	0.81		
		9.21	n.d.[d]		

[a] DS – degree of substitution, cellulose **1** was used for the synthesis; [b] the pH was measured in the sample solution; [c] average amount of positive charges from three measurements; [d] n.d. – not detectable.

Deprotonation of tetraalkylammonium ions does not occur. By contrast, a model for the ester substituent, (3-carboxypropyl)trimethylammonium chloride (CPTAC), was examined and no charges were determined by polyelectrolyte titration. Therefore, the results of the experiment with sample **44** suggest that another phenomenon was occurring beside deprotonation of the samples at increasing pH value.

3.2.2. Charging behaviour studied by potentiometric titration

Samples were subjected to potentiometric titration in order to study the protonation/deprotonation of the nitrogen-containing moieties. The samples titration was carried out in the presence of solution of potassium chloride (KCl, 0.1 M) as a neutral salt to prevent an unequal distribution of the mobile ions between the polymer chains and the external solution caused by Donnan equilibrium [103]. A glass electrode (Ag/AgCl) was used to measure the electrical potencial difference in the solution. The experiments were performed by addition of NaOH/KCl (forward titration) and/or HCl/KCl (backward titration) solutions at two different speeds. Slow titration (dE/dt = 0.1 mV/50 s) was used in most experiments and a fast titration (dE/dt = 0.1 mV/10 s) was used to determine the pK of amino group. The pKa was experimentally determined from the charging isotherm of forward titration performed in 8 min and calculated by nonlinear least squares fitting according to the literature [104]. The charging isotherm normalized to the mass of the cellulose-4-[*N*-methylammonium]butyrate chloride **11** indicated one deprotonation step within the range of $7 < pH < 11$ (Fig. 33).

The amino groups of cellulose-4-[*N*-methylammonium]butyrate chloride had a pK_a value of 9.54, which is much lower than the values published for compounds containing secondary amino groups like for example diethylamine (pKa = 10.8) [105]. Anticipating our further results, this is a consequence of the presence of two different species in the mixture (cellulose-4-[*N*-methylammonium]butyrate chloride and 4-[methylammonium]butyric acid chloride), which is

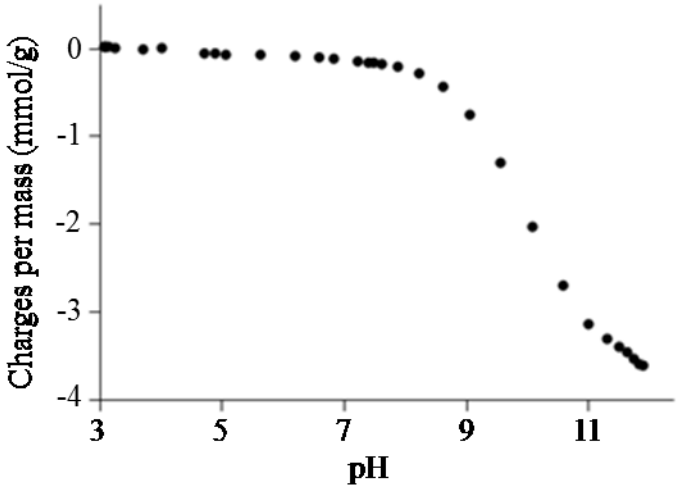


Fig. 33. Charging isotherm (forward titration) of cellulose-4-[*N*-methylammonium]butyrate chloride **11** (degree of substitution of 1.01) dissolved in aq. 0.1 M KCl.

formed as the product of the ester cleavage. It should be noted that the curve show a negative charge due to the Cl^- that is present as counterion in cellulose-4-[*N*-methylammonium]butyrate chloride, and which could not be experimentally quantified by the same technique.

The amount of protonated amino groups determined from the charging isotherm of the forward titration shown in Fig. 33 is 3.58 mmol/g, which is lower than the value determined by polyelectrolyte titration, that is 3.94 mmol of positive charges per gram at pH = 2.04 (Table 5) due to the reason mentioned above and also due to the error range between both methods.

In order to underpin the negative value of charges per mass (-3.58 mmol of positive charges per gram of sample) indicated by the potentiometric titration curve (Fig. 33), a new degree of substitution (DS_{new}) was calculated for sample **11**. A presence of 3.58 mmol of nitrogen atoms was considered for this calculation. Thus, DS_{new} of 1.1 was calculated for sample **11**, which was slightly higher value comparing to the DS determined by elemental analysis (DS of 1.01). The difference between both results was assigned to error between both techniques. Thus, the negative value determined by potentiometric titration was taken as an absolute value (|-3.58| = 3.58) for this study.

3.2.3. Stability of cellulose-4-[*N*-methylammonium]butyrate chloride and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride

Precipitation occurred starting at pH 11 when the samples were titrated up to a pH value of 11.5 (forward titration). No redissolution was observed during backward titration from pH of 11.5 to 2.5. This fact suggested that irreversible phenomena such as saponification of ester moieties possibly occurs at higher pH values. For a detailed study, sample **11** was slowly titrated (*dE/dt* = 0.1 mV/50 s) from pH 5 to pH 7 (Fig. 34, a) and the forward and backward curves are coincident, as expected at lower pH value. The results are in agreement with the suggested deprotonation/protonation of amino groups, as indicated by polyelectrolyte titration (see Fig. 32). A difference between these curves occurs upon titration of the sample from pH 5 to pH 8 (Fig. 34, b). It is even more pronounced for the titration up to pH value of 9 (Fig. 34, c). Thus, in addition to deprotonation of the amino groups, changes in the structure of the sample appear. It is recommended that the cellulose-4-[*N*-methylammonium]butyrate is used in solutions with pH ≤ 7 in order to prevent/deter saponification.

Although the ester substituent of sample **44** has no acidic hydrogen for deprotonation, it was also titrated and the same separation between forward and backward curves was identified. It is suggested that the ester bonds in the cellulose-4-[*N*-methylammonium]butyrate chlorides and cellulose-4-[*N,N.N*-trimethylammonium]butyrate chloride samples were cleaved in alkaline medium starting at pH = 8, which would yield cellulose and 4-[methylamino]butyric acid and (3-carboxypropyl)trimethylammonium chloride, CPTAC, as respective products (see Fig. 35).

ATR-FTIR analysis was performed on the isolated precipitate from sample **11** titrated up to pH 11.5 and compared to the spectrum of cellulose **1** (Fig. 36). Only the typical signals [100] of the cellulose backbone were observed, which indicated that hydrolysis occurred.

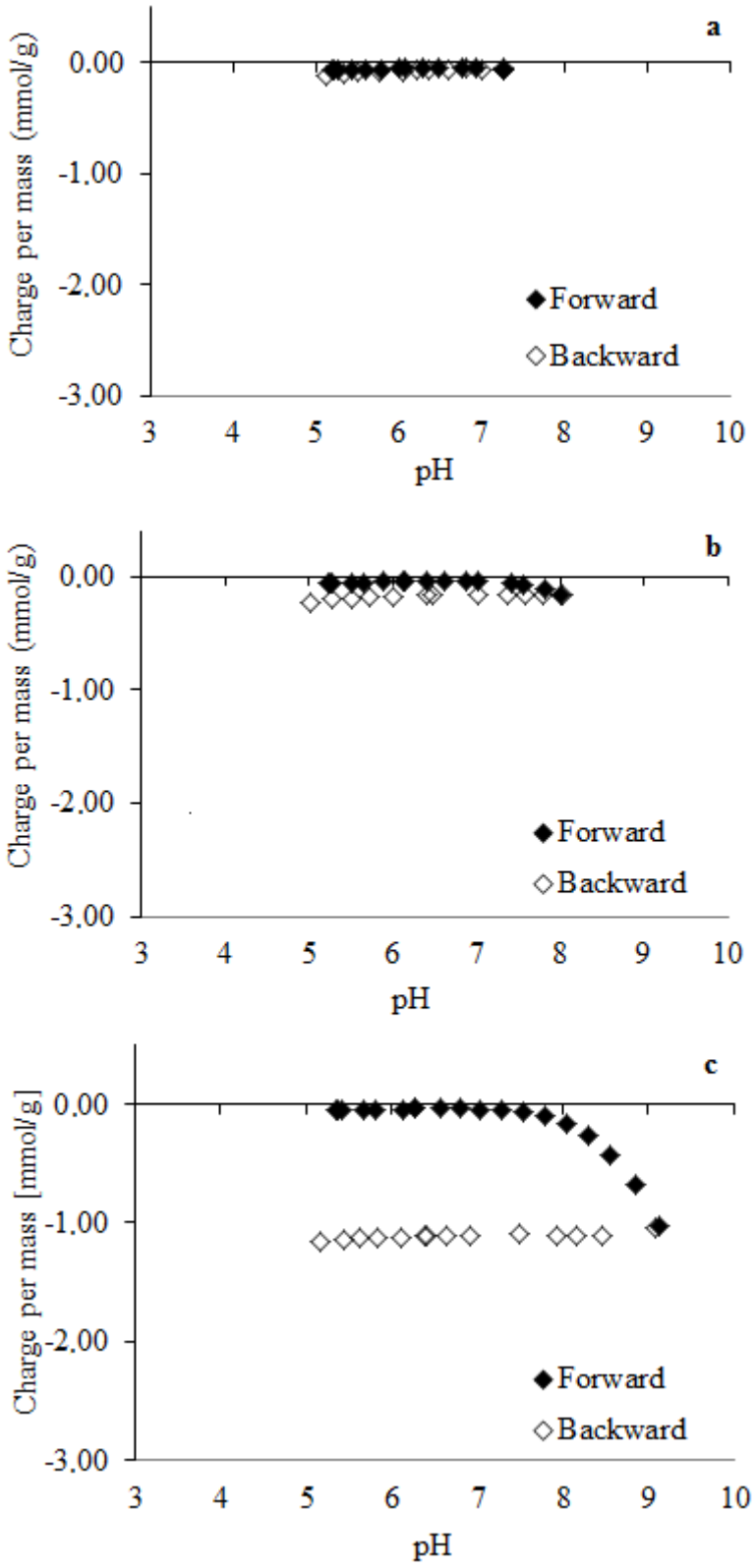


Fig. 34. Charging isotherms of cellulose-4-[*N*-methylammonium]butyrate chloride **11** (degree of substitution of 1.01, 0.1%, w/v) analysed in the ranges of 5 < pH < 7 (a), of 5 < pH < 8 (b), and of 5 < pH < 9 (c) by potentiometric titration.

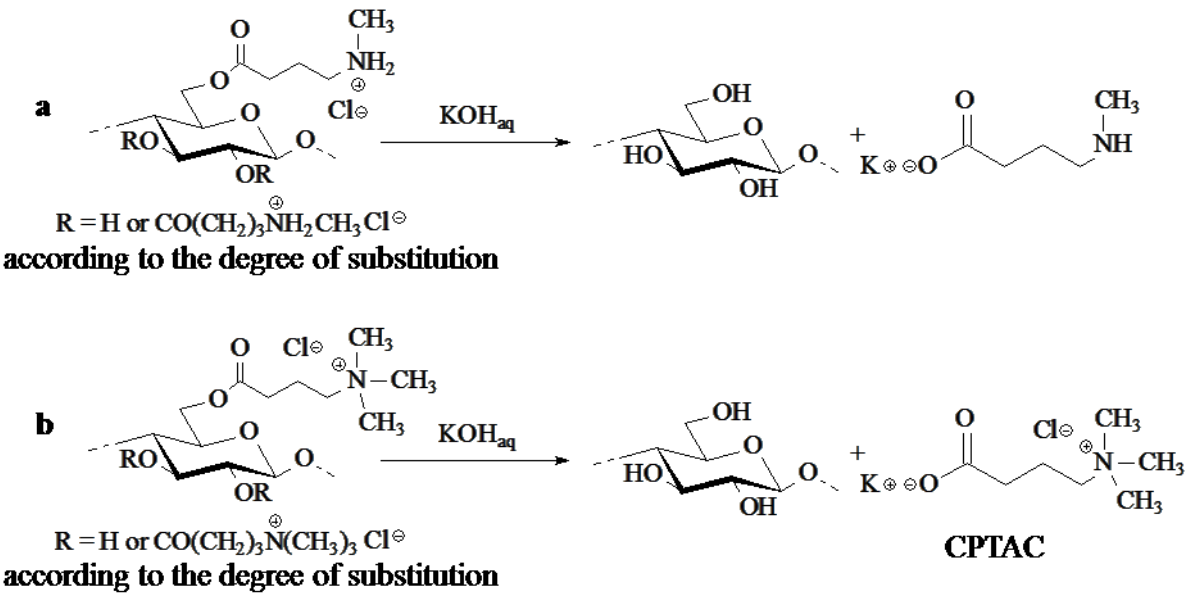


Fig. 35. Hydrolysis of the ester moiety of cellulose-4-[*N*-methylammonium]butyrate chloride (a) and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride (b) yielding regenerated cellulose and 4-[methylamino]butyric acid and (3-carboxypropyl)trimethylammonium chloride (CPTAC) as products, respectively.

Moreover, the total organic carbon and nitrogen content (TOC/TN) were measured in the solution of sample **11** before and after precipitation. Aliquots were taken at pH = 7 in the forward titration (aliquot A, Table 6) and again at the same pH value during the backward titration (aliquot B, Table 6). A strong decrease of carbon was found in **11** sample solution during backward titration from pH 11.5 to pH 7, indicating that pure cellulose had been precipitated (Table 6). The value for nitrogen indicated that nitrogen had remained in the solution.

In addition, positive charges were detected only in aliquot A (Table 6). Thus, the precipitated material is cellulose and the charged-ammonium groups were removed from the polymer backbone. The polyelectrolyte titration under formation of polyelectrolyte complexes between anionic and cationic polymers is mainly driven by electrostatic interactions, involving the increase in entropy, which results from the release of a large amount of water (desolvation) and

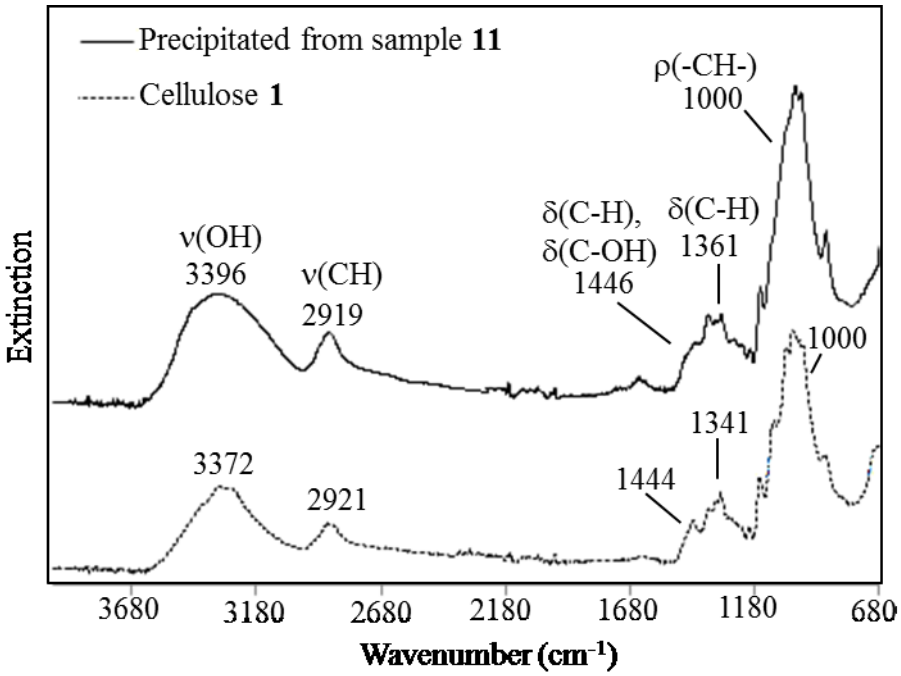


Fig. 36. ATR-FTIR analysis of the isolated precipitate generated from sample **11** at alkaline titration up to pH 11.5 and of the neat cellulose **1**.

counterions as the polycation and polyanion associate. Hence, polyelectrolyte titration of oppositely charged polyelectrolytes occurs while low molecular weight charged molecules cannot be detected by this method [106].

Table 6. Carbon, nitrogen, and content of positive charge in cellulose-4-[*N*-methylammonium]butyrate chloride **11** (degree of substitution of 1.01) potentiometrically titrated.

Aliquot	C (mmol/g)[c]	N (mmol/g)[c]	Positive charges (mmol/g)[d]
A (2.5 < pH < 7)[a]	70.3	2.9	1.21
B (11.5 < pH < 7)[b]	45.9	3.4	n.d.

[a] No precipitation; [b] precipitate was isolated; [c] determined by TOC/TN; [d] determined by polyelectrolyte titration, n.d.- not detectable.

3.2.3.1. Titration of 4-[methylammonium]butyric acid chloride

Deesterification of cellulose-4-[*N*-methylammonium]butyrate chloride results in the release of 4-[methylamino]butyric acid into the solution. Potentiometric titration of 4-[methylammonium]butyric acid chloride (MABA), synthesized in this work, was performed in order to simulate and further investigate the hydrolysis and release of 4-[methylamino]butyric acid. No precipitation was observed when the carboxylic acid was titrated. The titration curves are shown in Fig. 37. A difference was only observed in the backward titration curve of cellulose-4-[*N*-methylammonium]butyrate chloride (sample **11**, Fig. 37a).

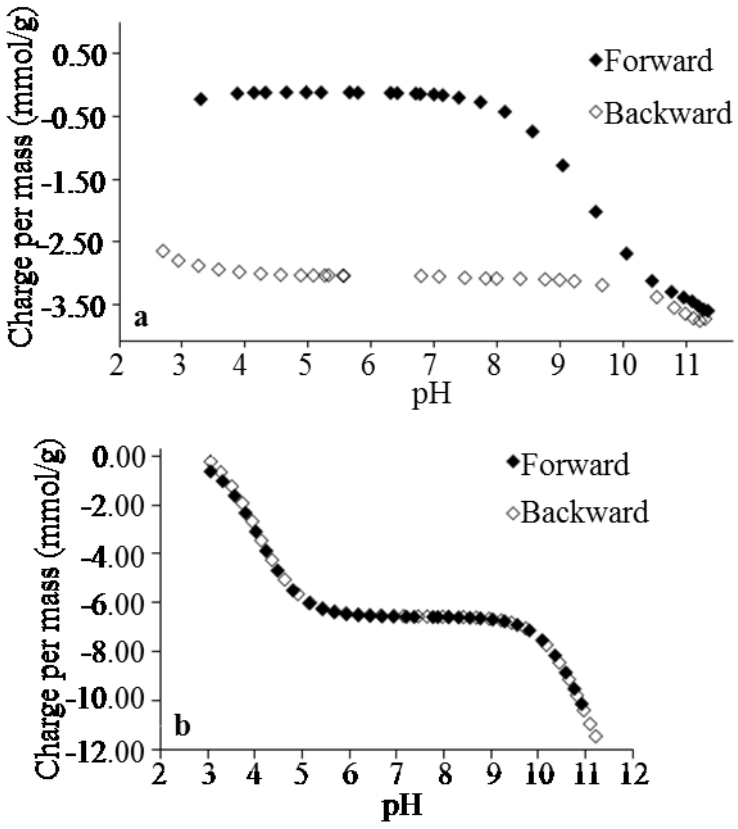


Fig. 37. Charging isotherm of cellulose-4-[*N*-methylammonium]butyrate chloride **11** (degree of substitution of 1.01, 0.1%, w/v, a) and 4-[methylammonium]butyric acid chloride (0.1%, w/v, b) determined by potentiometric titration.

The deprotonation of MABA ammonium groups in the pH range from 8 to 11.5 (Fig. 37b) shows a similar curve compared to the forward titration curve of cellulose-4-[*N*-methylammonium]butyrate chloride. The deprotonation of the carboxylic acid moieties and amino groups were clearly evidenced at pH = 4 and pH = 10.5, respectively. The dissociation constants of $pK_1 = 4.1$ (carboxylic acid moiety) and $pK_2 = 10.85$ (amino group) were calculated for this compound. Importantly, pK_2 value is now very close to the value expected for a secondary amine, and supposedly it is slightly lower than expected due to internal salt formation. From the potentiometric analysis of cellulose-4-[*N*-methylammonium]butyrate chloride (sample **11**) and MABA, it may be concluded that the deprotonated 4-[methylamino]butyric acid is released by hydrolysis of the cellulose ester (cellulose-4-[*N*-methylammonium]butyrate chloride) at pH > 8. In the backward titration curve of sample **11** (Fig. 37a), there is a clear step in the range of 2.6 < pH < 3.3. It may be assumed that 4-[methylamino]butyric acid was back titrated in a very small amount.

If dissociated carboxylic acids are present, the electrostatic interactions between oppositely charged groups may occur, too. It is therefore supposed that some of the *in situ* generated 4-[methylamino]butyric acid interacts with the cationic amino groups by salt formation, thus the carboxylic group cannot be protonated and separation between forward and backward curves occurred. Hydrolysis of the ester groups in cationically modified polyacrylamide at pH 8.5 was observed by, among others, Murphy *et al.* [107]. It is known that acrylate groups are formed during the hydrolysis of cationic polyacrylamides and they can also form an internal salt with the cationic trimethylaminoethyl groups [108]. Regarding the peptide bonds, studies by potentiometric titration stated that the binding of α-aminoacids by tetrasulfonatomethylcalix-4-resorcinarene was driven by electrostatic interactions and hydrogen-bondings, and a complex dependence on pH is formed [109]. More evidence of salt formation between 4-[methylamino]butyric acid *in situ* generated and cellulose-4-[*N*-methylammonium]butyrate

chloride was not collected for this thesis and further studies are required to demonstrate this unequivocally.

3.2.3.2. Hydrolysis study

Additional information was obtained by polyelectrolyte titration analysing the amount of protonated amino groups as a function of time as well as by ATR-FTIR spectroscopy. Aqueous solutions of cellulose-4-[*N*-methylammonium]butyrate chloride (sample **11)** at pH values of 3, 6, 7, and 8 were constantly shaken at room temperature and analysed at after 30 min, 1 h, 2 h, 6 h, 12 h, 24 h, 48 h, 1, 2, 3, and 4 weeks.

Results of polyelectrolyte titration clearly show that the amount of positive charges at pH up to 7 remained unchanged (Fig. 38).

Sample **11** possesses an average value of 4.02 ± 0.2 mmol of positive charges per gram of sample at pH = 3, 3.67 ± 0.1 mmol/g at pH = 6, and 3.49 ± 0.2 mmol/g at pH = 7. Thus, the cellulose derivative remains intact and positively charged in an aqueous solution at pH ≤ 7 for at least 1 month. The ester moiety is stable at acidic pH and the amino group remains fully protonated. However, cellulose-4-[*N*-methylammonium]butyrate chloride (sample **11**) dissolved in aqueous alkaline medium (at pH = 8, 3.24 mmol/g) shows slowly decreasing positive charges within 2 h (3.21 mmol/g) that is more pronounced after 6 hours (2.87 mmol/g, Fig. 38). After 24 hours, 2.33 mmol/g of positive charges are determined. After 1 week of storage at alkaline pH, less than 50% of the initial charges could be determined (1.32 mmol/g). After 28 days of storage, the sample solution contains only 0.71 mmol of positive charges per gram of sample. A decrease of pH value is also noted during this experiment (from pH = 8 to pH = 6). Deprotonation of amino groups and slow cleavage of the ester moieties take place at the same time, leading to the loss of positive charges. The deprotonation of amino moieties could not be studied in detail due to the instability of the samples at alkaline pH values. The decrease in pH may be attributed to

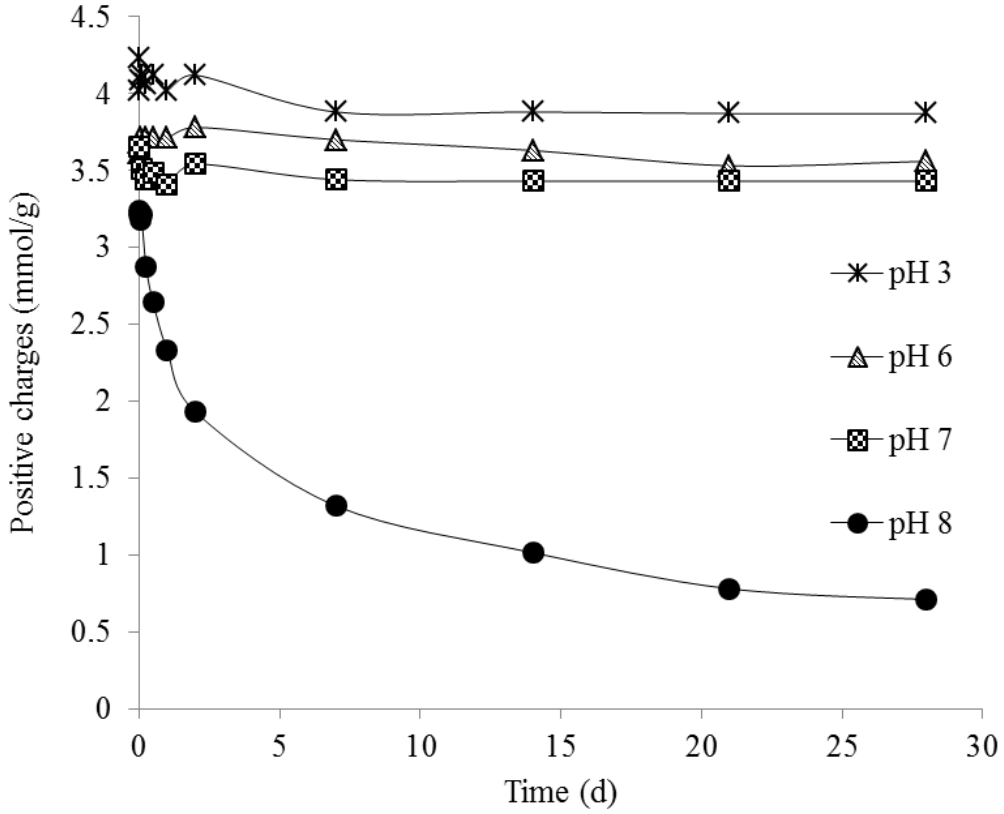


Fig. 38. Amount of positive charges (mmol/g) bonded to polycations of cellulose-4-[*N*-methylammonium]butyrate chloride **11** (degree of substitution of 1.01) in aqueous solution at different pH values measured for a period of up to 28 days by polyelectrolyte titration.

the generation of carboxylic acid species resulting from the cleavage of the esters over time.

ATR-FTIR analyses and polyelectrolyte titration were simultaneously performed in order to obtain further evidence of the ester cleavage. A sample (1 mL) was taken from the solutions and dried by nitrogen flow to form thin films. The ATR-FTIR spectrum of a film obtained from cellulose-4-[*N*-methylammonium]butyrate chloride, sample **11,** stored for 21 days at pH = 3, shows the typical signals of cellulose-4-[*N*-methylammonium]butyrate chloride (Fig. 39, a). The strong and broad signal at 3384 cm^{-1} indicates the presence of hydroxyl groups as typical for samples with a DS below 3. The amino groups could not be clearly identified in the spectrum because their signal appears in the same range as the signal of the strong OH signal. Signals around 2900 cm^{-1} are attributed to aliphatic CH-vibrations. The signal at 1735 cm^{-1} (νC=O) is

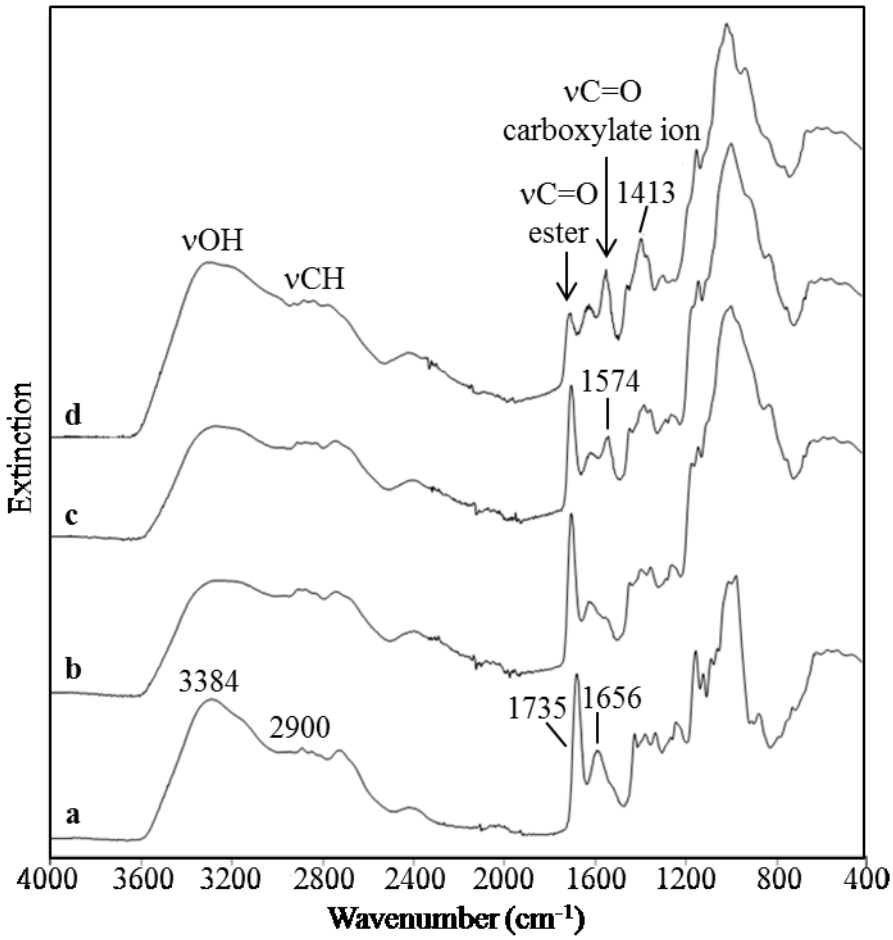


Fig. 39. ATR-FTIR spectra of cellulose-4-[*N*-methylammonium]butyrate chloride sample **11** (degree of substitution of 1.01) at pH 3 after 21 days (a) and at pH 8 after 2 hours (b), 1 day (c) and, 28 days (d). Films of dried solution were measured.

characteristic of the ester moiety, while the signal at 1656 cm^{-1} is attributed to the OH bending signal of water (1653-1640 cm^{-1}) [107, 110].

The ATR-FTIR spectra of the films obtained from a solution of pH = 8 showed that the signal at 1735 cm^{-1} (νC=O) decreased with time. Increases in a new signal at 1574 cm^{-1} and at 1413 cm^{-1} were observed after 2 hours (Fig. 39). A typical shift of the signal at 1732 cm^{-1} to 1583 cm^{-1} (and 1418 cm^{-1}) for carboxylate ions (COO^-, asymmetric and symmetric stretch) was found [111]. This indicated that hydrolysis of the ester group and consecutive formation of carboxyl groups

occur in the sample solution at pH = 8 after 2 hours (decrease of 1% in positive charges). The sample was subjected to pH = 12 and the signal at 1735 cm^{-1} ($\nu C{=}O_{ester}$) disappeared entirely.

The presence of carboxyl groups was also investigated by Raman spectroscopy. As shown in Fig. 40a, the Raman spectrum of cellulose-4-[*N*-methylammonium]butyrate chloride, sample **11**, originally exhibits signals at 2936, 1456, 1103 and 905 cm^{-1}, which represent the stretching (ν, symmetric) and deformation (δ, asymmetric) vibrations of CH-, CH_2-, CH_3-moieties and carbon-carbon bonds. The signal at 1744 cm^{-1} is assigned to the ester moiety [112]. When cellulose-4-[*N*-methylammonium]butyrate chloride, sample **11**, is dissolved in water and the pH is adjusted to 12, a precipitate was formed that was isolated (without washing), freeze-dried and analysed by Raman spectroscopy (Fig. 40, b). The shift of carbonyl signal from 1744 cm^{-1} to 1686 cm^{-1}

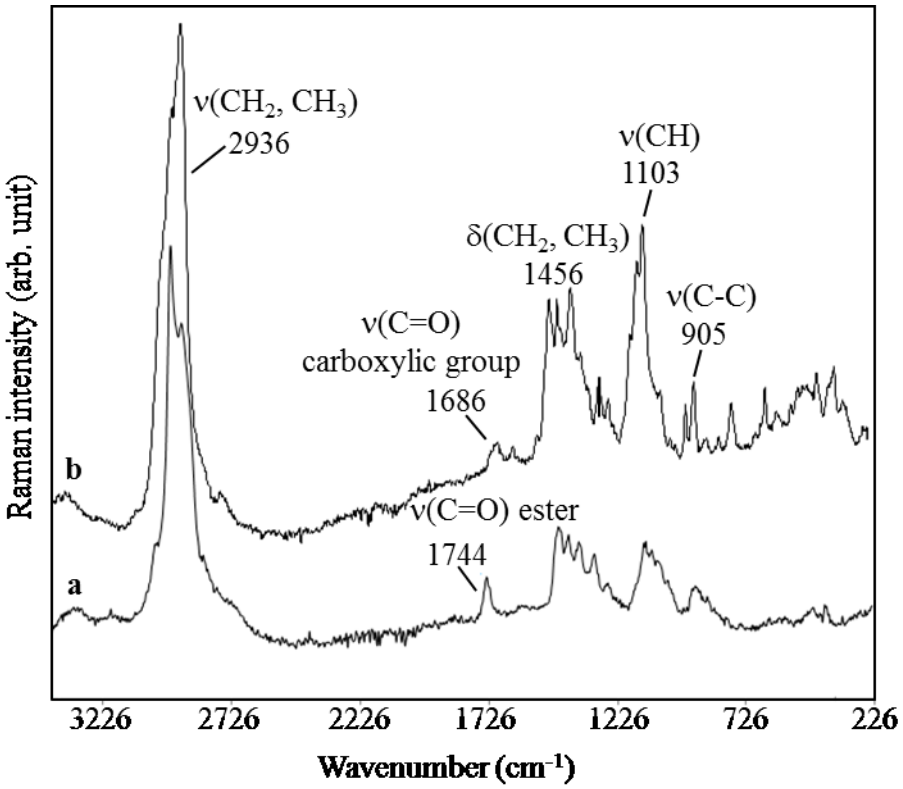


Fig. 40. Raman spectra of cellulose-4-[*N*-methylammonium]butyrate chloride (sample **11**, degree of substitution of 1.01, a) and of the freeze-dried precipitate formed after the pH of the solution was adjusted to pH 12 (b). Arb. unit, arbitrary unit.

indicates that the precipitate consisted in a mixture of regenerated cellulose and carboxyl groups. In other words, the carboxyl groups were generated by hydrolysis of the ester moiety and the corresponding low molecular acid is formed.

The combination of polyelectrolyte- and potentiometric titrations were useful methods to characterized the main property of cellulose-4-[*N*-methylammonium]butyrate chloride and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride: 'cationic' polyelectrolytes [113]. Due to stability of cellulose-4-[*N*-methylammonium]butyrate chloride in aqueous solution at pH ≤ 7, some applications can be suggested for this compound. Its use as flocculants or thickener seems to be interesting because degradation may be induced after its application.

3.3. Properties of cellulose-4-[*N*-methylammonium]butyrate chloride, hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride

Understanding of the charging behavior of cellulose-4-[*N*-methylammonium]butyrate chloride and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride is important for the study of further properties for the synthesized compounds. The investigations were carried out in aqueous solutions at different concentrations.

3.3.1. Viscosity measurements

3.3.1.1. Influence of storage time on stability of cellulose-4-[*N*-methylammonium]butyrate chloride determined by capillary viscosimetry

To evaluate the stability of cellulose-4-[*N*-methylammonium]butyrate chloride (sample **11**, 0.4%, w/v) upon storage in aqueous solution at pH 2, 7, and 9 (Fig. 41) over a range of 63 days,

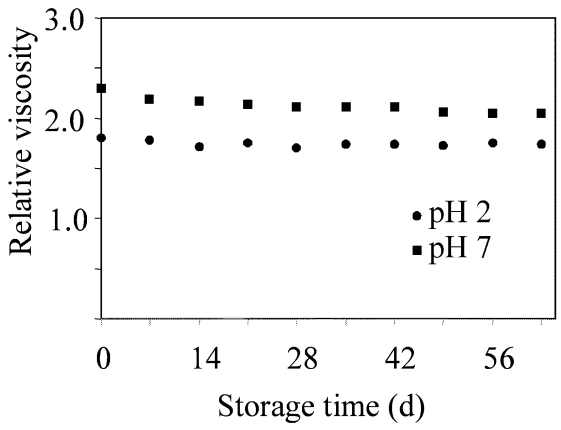


Fig. 41. Relative viscosity of cellulose-4-[*N*-methylammonium]butyrate chloride (sample **11**, degree of substitution of 1.01, 0.4%, w/v) stored at pH 2 and 7 during 63 days.

the relative viscosity was determined by capillary viscometry. At pH 9, the sample solution became cloudy very fast after the first measurement (day one) probably due to the deprotonation of ammonium groups and saponification of the ester moiety as explained in the previous chapter. In contrast, there was almost no change in relative viscosity (η/η_0) of samples stored in water at pH 2 and 7 and the samples exhibited good storage stability. Decreasing viscosity with increasing time was found for aqueous solutions of poly(acrylamide) in the literature [114, 115]. A change in the conformation of the polymer coils was assigned for the decrease of the material functions with increasing storage time.

3.3.1.2. Comparison of intrinsic viscosity of hydroxyethyl cellulose, hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride and polymer JR400 in water and in aq. 0.1 M $NaNO_3$

The intrinsic viscosity [η] reflects the behavior of the macromolecules in the corresponding solvent. The hydrodynamic volume occupied by a macromolecule is affected by the medium in which the polymer is dissolved. The viscosity depends not only on the molar mass but also on the type of substituent (ionic or non-ionic) as well as on the distribution of the substituents.

Reduced viscosity of aqueous sample solutions was measured at different concentrations. Solutions of hydroxyethyl cellulose (MS of 3.66) and hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride (sample **24**, DS of 0.46, see Table 2) in aq. $NaNO_3$ (0.1 M) and in water were measured by capillary viscometry at 20°C (Fig. 42). The intrinsic viscosity of polymer JR 400 (cellulose-2-(2-hydroxy-3-(trimethylammonium)propoxy)ethylether chloride, commercial product, Fig. 43) was determined as well in aq. $NaNO_3$ (0.1 M) using the same conditions in order to compare the results. The intrinsic viscosities were measured in a concentration range up 3 mg/mL.

A value of $[\eta]$ = 219 mL/g was determined for hydroxyethyl cellulose dissolved in water (Fig. 42, HEC). The viscosity of such compound dissolved in aq. $NaNO_3$ (0.1 M) did not show a large difference ($[\eta]$ = 319 mL/g) due to the fact that it is a non-ionic macromolecule. Addition of ionic groups in the polymer chain and an increase in the degree of substitution increase the rigidity of polymer structure. An increasing rigidity decreases the ability of the chain to form a random coil and leads to an increased intrinsic viscosity [117]. For this reason, a higher value of $[\eta]$ = 1574 mL/g was determined for sample **24** (DS of 0.46) dissolved in water. The polymer coil of sample **24** is expanded due to the coulomb forces between the ionic groups. The concentration of counterions inside of the polymer coil is higher than outside and the osmotic pressure forces solvent into the coil leading to an expansion [117]. The addition of salt ($NaNO_3$, 0.1 M) to a **24** sample solution compensates the effects of the osmotic pressure as well as the coulomb forces by shielding the dissociated ionic groups. Then, a low value of $[\eta]$ = 409 mL/g was determined for sample **24** dissolved in saline solution, which was similar to the value determined for the HEC ($[\eta]$ = 319 mL/g, non-ionic polymer). Therefore, the values of intrinsic viscosities in salt free solution do not reflect coil expansion. A value of $[\eta]$ = 426 mL/g was determined for polymer JR400 in aq. $NaNO_3$ (0.1 M) which is similar to the intrinsic viscosity of

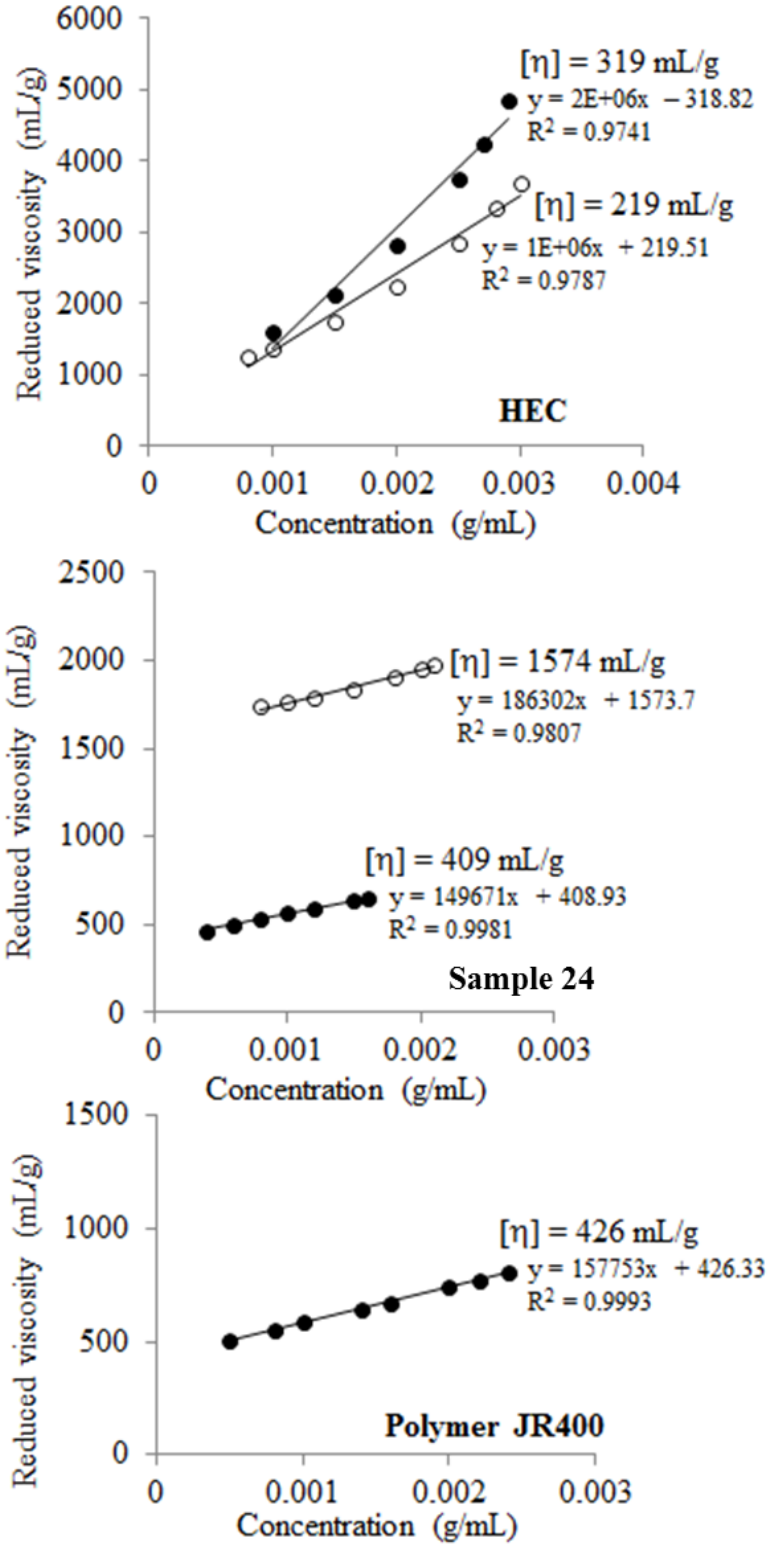


Fig. 42. Correlation between reduced viscosity and concentration of hydroxyethyl cellulose (HEC), hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride (sample **24**, DS of 0.46) and polymer JR 400, measured by capillary viscometry in water (○) and in aq. $NaNO_3$ (●, 0.1M).

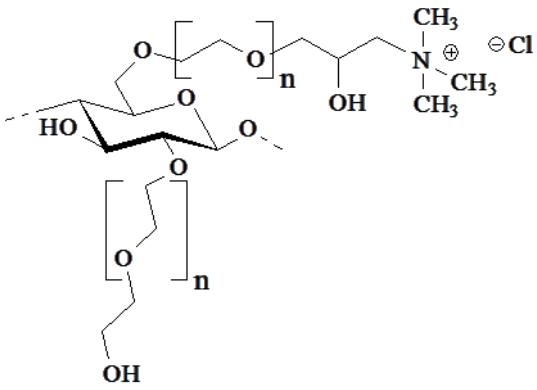


Fig. 43. Structure of cellulose-2-(2-hydroxy-3-(trimethylammonium)propoxy)ethylether chloride (polymer JR400), commercially acquired [116].

sample **24** ([η] = 409 mL/g). Due to the fact that the viscosities of polymer JR400 and sample **24** are comparable, the study of hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride as viscosifier in personal care products [118] could be an object of interesting study.

Comparisons between intrinsic viscosities of cationic polysaccharides dissolved in water and in saline solution also can be found in the literature [119].

Molar mass and dimension of a single polymer coil (polymer conformation) can be correlated to the intrinsic viscosity of the samples and determined by Kuhn-Mark-Houwink-Sakurada-relationship [120]. However, this research was not carried out in the present work and more studies are required.

3.3.1.3. Shear viscosity of cellulose-4-[*N*-methylammonium]butyrate chloride, hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride with increasing shear rate

The dynamic viscosity of ammonium cellulose esters dissolved in water and in NaCl solution with increasing shear rate was studied at 20°C. The viscosity curve of any ammonium cellulose ester solution, as shown in Fig. 44, dropped drastically with increasing shear rate which

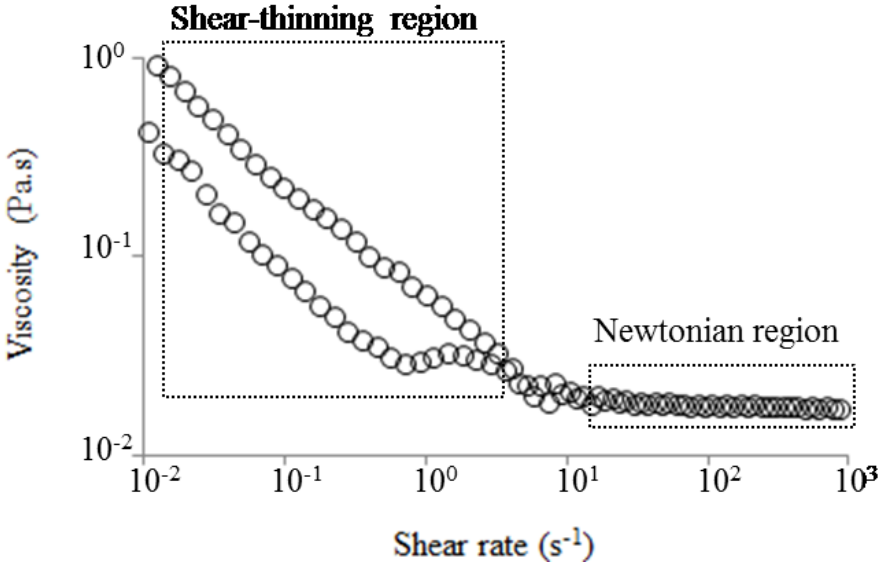


Fig. 44. Typical viscosity curve of cellulose-4-[*N*-methylammonium]butyrate chloride (sample **9**, 2%, w/v, in aq. 0.1 M NaCl) determined by rheometer at 20°C.

corresponds to a pseudoplastic-type fluid. This curve is typical for emulsions, dispersions and suspensions of commercially important cellulose derivatives [121]. Two regions were assigned in viscosity curve of the ammonium cellulose esters: a region of shear-thinning behaviour at lower shear rates and a region of Newtonian behaviour at higher shear rates. The similarity with Newtonian liquids is characterized by a defined viscosity, independently on shear rate.

A hysteresis caused by shear stress from Brownian motion of macromolecules was observed in the shear-thinning from higher to lower shear rate. In the Newtonian region, the shear effect in the solution was reversible indicating that an optimum of perfect orientation of macromolecules has been reached [122, 123].

In order to simplify the results obtained in this work, only viscosity curves of sample solutions obtained from lower to higher shear rates will be presented and a comparison of the viscosities will be performed with respect to the regions of Newtonian behavior (shear rate = 10 - 10^3 s^{-1}).

Shear viscosity of cellulose-4-[*N*-methylammonium]butyrate chloride in water and in aq. NaCl solution

In the first series of experiments, the flow curve of a 2% (w/v) cellulose-4-[*N*-methylammonium]butyrate chloride solution (sample **9**, DS of 0.86) was measured in neat water and in aqueous solutions of NaCl at different concentrations (from 0.05 M to 0.5 M, Fig. 45). Cellulose-4-[*N*-methylammonium]butyrate chloride (sample **9**) presented a high viscosity in aqueous solution (88.6 Pa.s, 2%, w/v). Its viscosity decreases with increasing of saline concentration: viscosity values of 0.080 Pa.s, 0.017 Pa.s, 0.013 Pa.s and 0.012 Pa.s were determined for the sample dissolved at aq. 0.05 M, 0.1 M, 0.2 M and 0.5 M of NaCl, respectively. The decreasing viscosity was expected by addition of salt to a cationic cellulose solution due to the same reasons mentioned in **3.3.1.2**. Similar behavior is reported for sodium pectinate solutions at different NaCl concentrations [124].

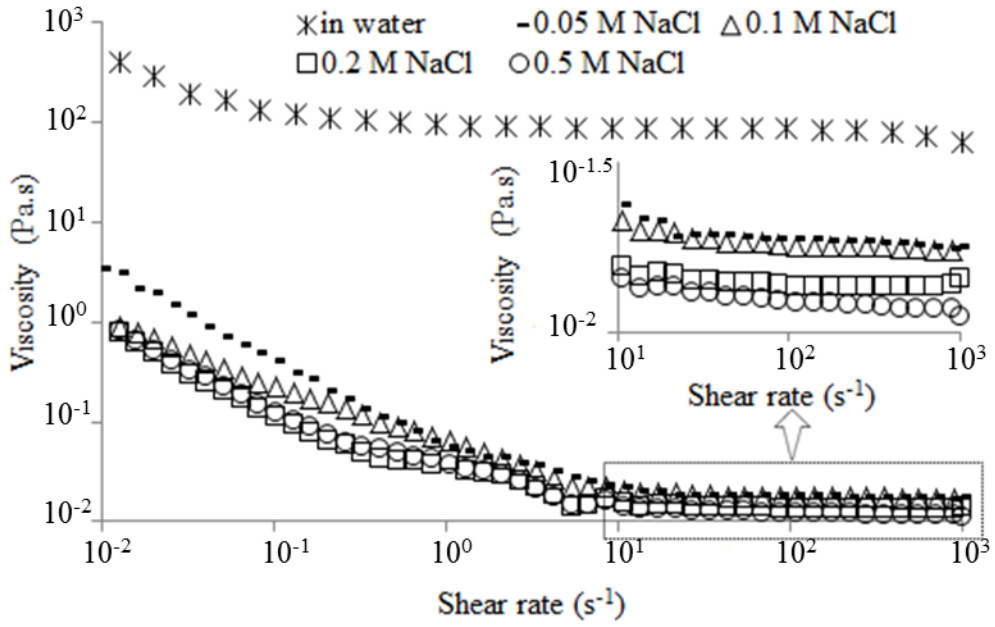


Fig. 45. Flow curves of cellulose-4-[*N*-methylammonium]butyrate chloride (2%, w/v, sample **9**, DS of 0.86) dissolved in neat water and in aq. 0.05 M, 0.1 M, 0.2 M and 0.5 M NaCl.

Using a NaCl concentration of 0.1 M, the effect of increasing concentration of cellulose-4-[*N*-methylammonium]butyrate chloride (sample **9**) in solution was investigated (Fig. 46).

The viscosities increased by increasing sample concentration in the solution. The increase of concentration resulted in the increase of polymer chains, leading to the interactions or entanglements, and restriction of the motion of individual chains [125]. Comparable results are reported for carboxymethyl cellulose of different degree of polymerization in aq. 0.1 M NaCl [126].

Flow curves of cellulose-4-[*N*-methylammonium]butyrate chlorides with different DS and DP in aq. 0.1 M NaCl (2%, w/v) were measured (Fig. 47).

The derivative of higher DP (sample **9**, DP_n 415, 0.017 Pa.s) presented the highest viscosity compared to derivatives of lower DP (sample **8**, DP_n 155, 0.008 Pa s). Moreover, the viscosity

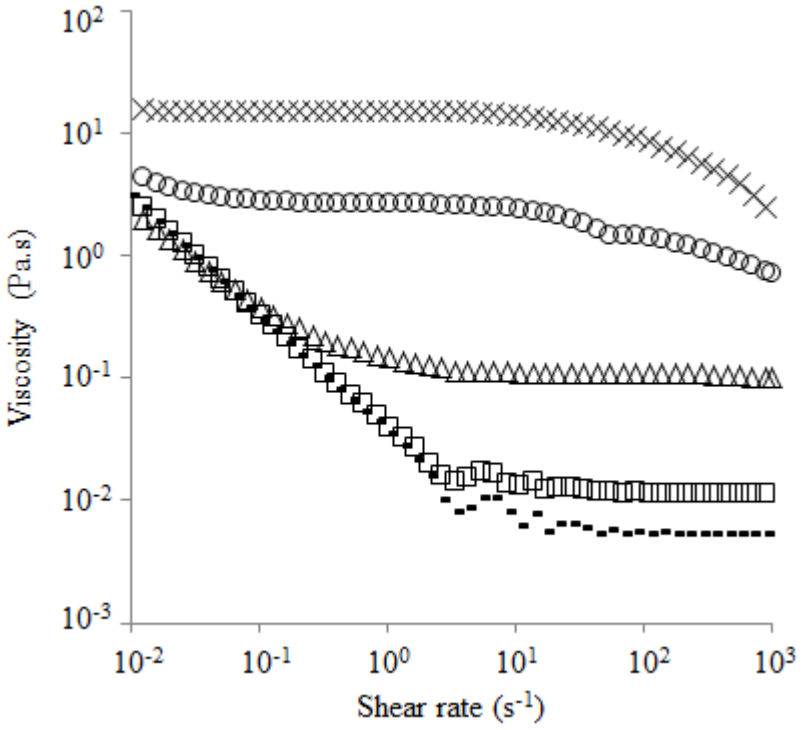
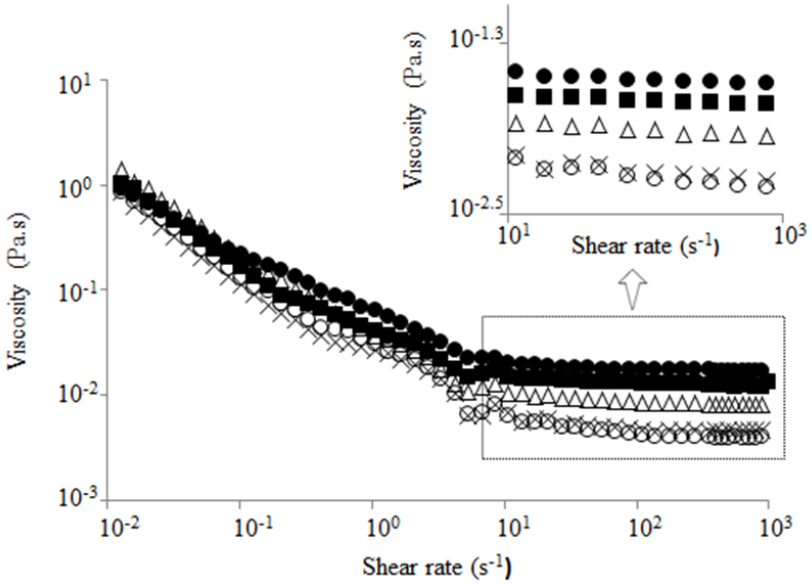


Fig. 46. Flow curves of cellulose-4-[*N*-methylammonium]butyrate chloride (sample **9**, DS of 0.86) dissolved at concentrations of 1% (-), 2% (□), 5% (Δ), 10% (○) and 20% (X, w/v) in aq. 0.1 M NaCl.



Symbol	Sample	DS	DP
●	**9**	0.86	415
Δ	**8**	0.85	155
■	**5**	0.31	99
X	**11**	1.01	109
○	**6**	0.62	65

Fig. 47. Flow curves of cellulose-4-[*N*-methylammonium]butyrate chlorides in aq. 0.1 M NaCl (2%, w/v) varying in DS and starting material at 20°C.

decreases with increasing DS for samples with comparable DP. An aqueous solution of cellulose-4-[*N*-methylammonium]butyrate chloride (sample **5**, DS of 0.31, DP_n 99, 0.012 Pa s) of lower DS presented higher viscosity than a sample of higher DS (sample **11**, DS of 1.01, DP_n 109, 0.005 Pa s). The stiffness of the polymer chain was not really influenced by the introduction of more ionic groups in the salt solution for cellulose-4-[*N*-methylammonium]butyrate chloride. Low viscosities were measured for samples with low DS and low DP (sample **6**, DS of 0.62, DP_n 65, 0.004 Pa s). Neat water was measured as a reference and presented a viscosity value of 0.001 Pa.s (curve not shown). Different solution media and different polymer concentrations may cause an expansion or contraction of the polymer coil in solution compared to the linear, flexible coil in its unperturbed dimensions. The cationic moieties along the polymer chain affect the viscosity of polymer solution. For high polymer concentrations, the amount of the counter ions inside the polymer coil is higher than outside and the osmotic pressure forces the solvent into the coil leading to an expansion. When a small concentration of the polymer in solution is measured, a decreasing osmotic pressure and a contraction of the coil occur [117]. In addition, stronger

charge delocalization within the polyelectrolytes can lead to weakening of molecular interactions and therefore lower solution viscosities [127, 128]. Moreover, the impact of the intra- and intermolecular hydrogen bonds must be taken into account, which influence the polymer–polymer interactions and, hence, the viscosity of the solution.

Flow curves of these samples in aqueous solution presented higher viscosities and were published elsewhere [91].

Viscosity of hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride

The flow curve of hydroxyethyl cellulose (HEC) and hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride (sample **24**, DS of 0.46) was measured in water and in aq. 0.5 M NaCl (Fig. 48). Their pseudoplastic curves did not present Newtonian behavior at increasing shear rate as typical flow curves of hydroxyethyl cellulose found in the literature do [129].

Similar viscosity curve was obtained for hydroxyethyl cellulose (1%, w/v) dissolved in water (0.19 Pa.s, 10^2 s^{-1}) and in aq. 0.5 M NaCl (0.17 Pa.s, 10^2 s^{-1}) as expected for a non-ionic macromolecule. At a comparable polymer concentration of 1% (w/v), a slightly lower viscosity value was determined for sample **24** (0.15 Pa.s, 10^2 s^{-1}). In general, higher viscosity was obtained for sample **24** dissolved in water compared to the sample dissolved in aq. 0.5 M NaCl due to the same reasons pointed out in **3.3.1.2**. Moreover, increasing sample concentration, the viscosity increases as expected. Flow curves of polymer JR400 (data not shown) were measured in aq. 0.5 M NaCl as well and the results were according to the literature [130]. The determined viscosities at shear rate of 10^2 s^{-1} are summarized in Table 7. Comparing to the viscosities measured for polymer JR400, sample **24** presented higher viscosity independently at all concentrations studied.

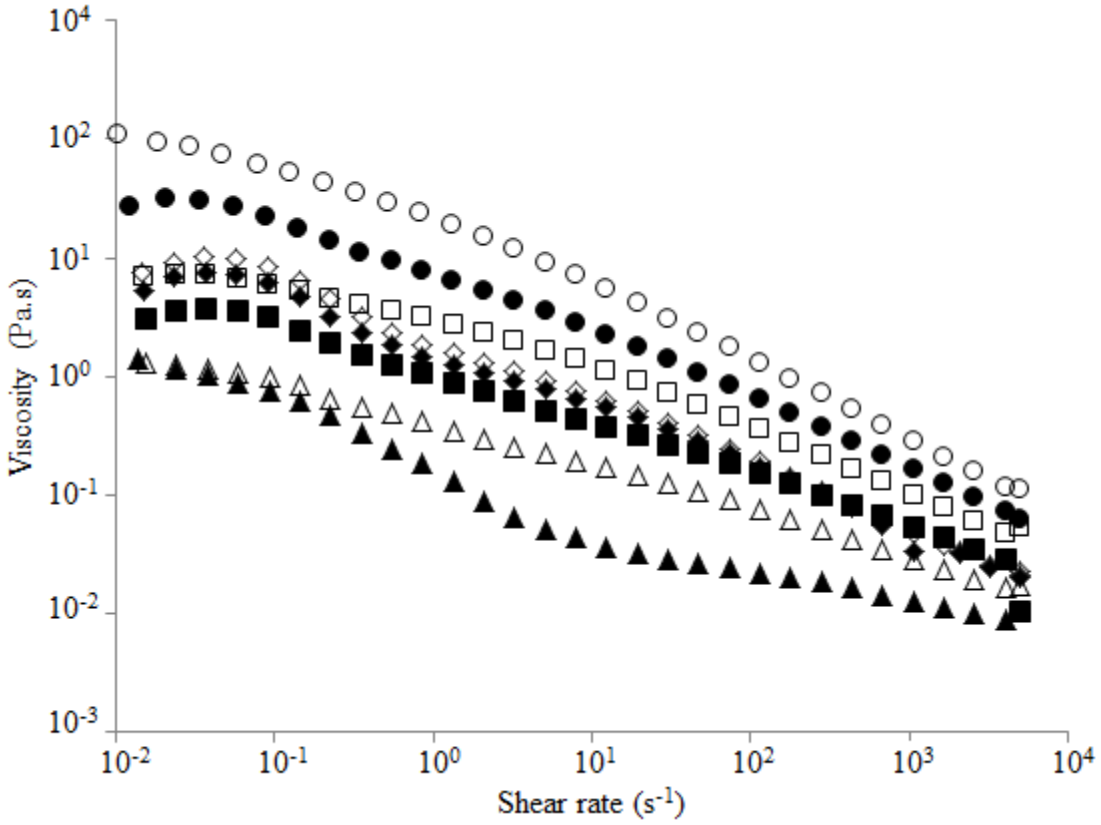


Fig. 48. Flow curves of hydroxyethyl cellulose (HEC, 1%, w/v, ◊) and hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride (sample **24**) measured in concentrations of 0.5% (Δ), 1% (□) and 2% (○, w/v) at 20°C. Samples dissolved in neat water (open symbols) and in aq. 0.5 M NaCl (filled symbols).

Table 7. Comparison between the viscosity of hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride (sample **24**) and polymer JR400 at shear rate of 10^2 s^{-1} determined by rheometer in aq. 0.5 M NaCl at 20°C.

Concentration of polymer (%) in aq. 0.5 M NaCl	Viscosity of polymer JR400 (Pa.s at 10^2 s^{-1})	Viscosity of sample **24** (Pa.s at 10^2 s^{-1})
0.5	0.010	0.022
1	0.038	0.150
2	0.221	0.644

Viscosity of cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride

The flow curve of different cellulose-4-[*N,N,N*-trimethylammonium]butyrate chlorides in aq. 0.5 M NaCl solution (2%, w/v) was measured (Fig. 49). For most of the solutions, a Newtonian behavior was observed over 2–3 decades of shear rates at 20°C. Derivative of higher DP (sample **47**, DP_n 124, 8 mPa s) presented the highest viscosity compared to derivatives of lower DP (sample **44**, DP_n 63, and **45**, DP_n 31, 3 mPa.s) at comparable DS. A solution of cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride (sample **49**, DS of 0.75, DP_n 18, 2 mPa.s) with lower DP and higher DS presented lower viscosity as well, which means that the viscosity of cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride solutions depends mostly on its degree of polymerization. The same viscosity behavior was observed in flow curves determined in aqueous solution of such samples (data not shown). The influence of cationic moieties on the viscosity was not studied in this work for cellulose-4-[*N,N,N*-trimethylammonium]butyrate

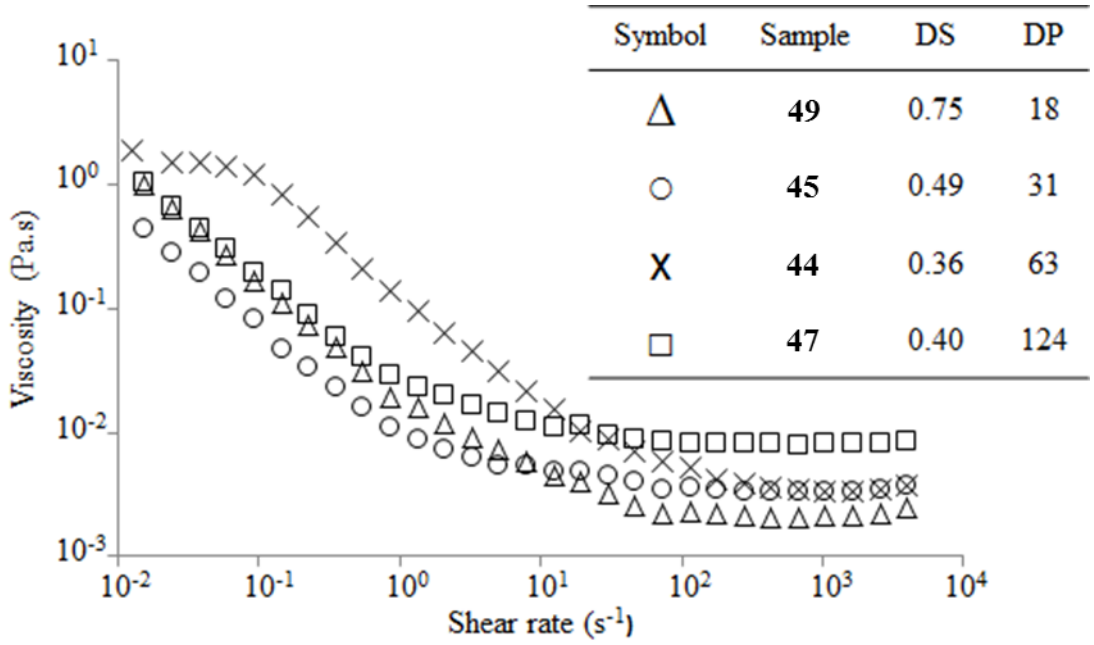


Fig. 49. Flow curves of cellulose-4-[*N,N,N*-trimethylammonium]butyrate chlorides (samples **44**, **45**, **47** and **49**, see Table 4), 2% (w/v) in aq. 0.5 M NaCl at 20°C.

chlorides. Further investigations in sample solutions at comparable DP and different DS are required to accomplish it.

This study was important to show that the rheological properties of cellulose and hydroxyethyl cellulose derivative solutions can be controlled by modifying the polymer backbone with ionic groups in order to increase or to decrease the viscosity.

3.3.2. Complex formation between polycationic cellulose derivatives (cellulose-4-[*N*-methylammonium]butyrate chloride, hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride) with various polyanions

Water-soluble polyelectrolytes possess the ability to form complexes in solution with a suitable 'counterpart' species. Polyelectrolyte complexes are colloidal structures that are governed by intermolecular forces (Coulombic interaction, van der Waals and hydrogen bond) [65]. The complex formation process is approached via the concept of coacervation complex formation [131]. This process depends on the concentration of the polyelectrolytes, charge density and on the solvent used. Generally, the particles are formed in a deviation from a 1:1 stoichiometry between cationic and anionic moieties [132]. Preliminary studies revealed the ability of the cellulose-4-[*N*-methylammonium]butyrate chloride, hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride to form complexes with polyanions. The particles were stable for at least 3 months in aqueous solution.

<u>Complex formation of cellulose-4-[*N*-methylammonium]butyrate chloride with carboxymethyl cellulose in water</u>

Aqueous solution of cellulose-4-[*N*-methylammonium]butyrate chlorides (sample **5**, DS of 0.31,

and **11**, DS of 1.01, both at 1% in solution, w/v) were dripped into an aqueous solution of carboxymethyl cellulose (CMC, DS of 0.95, 1% in solution, w/v). Comparatively soft particles were formed by the electrostatic interaction between the ammonium groups of sample **5** and the carboxyl moieties of CMC. A very smooth polyelectrolyte complex membrane was obtained (Fig. 50a). In the case of a lower concentration of aqueous CMC of 0.25% (w/v), the droplet of the polycation passes through the polyanion solution, while forming tubular structures instead of beads (Fig. 50b) due to the decreasing concentration of the polyanion. On the contrary, beads formed by the dropping of sample **11** (1%, w/v) into CMC solutions (1%, w/v, Fig. 50c) tend to collapse after few minutes. The sample **11** is more positively charged than the sample **5**, and here was observed that increasing the charge density of the polycation in the complex increases the swelling of the particles, thereby leading to their fast collapse [65]. The complex formation process was not optimized in this work. However, the use of different concentrations of polyelectrolyte cellulose derivatives may yield stable spheres as reported in the literature [133].

Fig. 51a shows cellulose-4-[*N*-methylammonium]butyrate chloride (sample **20**, DS of 0.63) dissolved in aq. 0.5 M NaCl dripped into cellulose sulfate solution (DS of 0.70, 1% in aq. 0.5 M

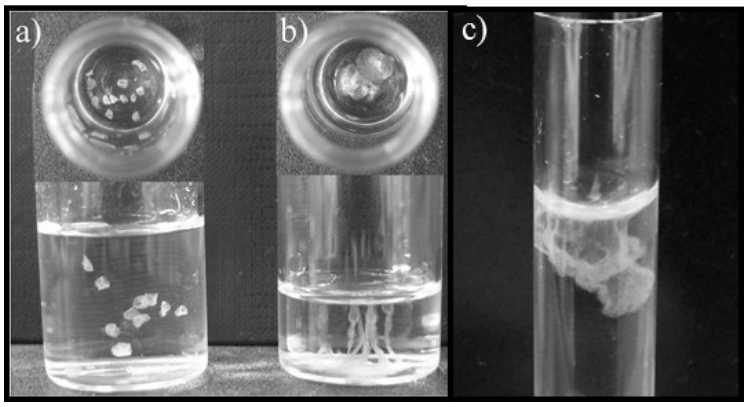


Fig. 50. Complex particles formed from aqueous solution of carboxymethyl cellulose (degree of substitution, DS, of 0.95) and droplets of aqueous solution of cellulose-4-[*N*-methylammonium]butyrate chlorides (sample **5**, DS of 0.31, a and b, and sample **11**, DS of 1.01, c).

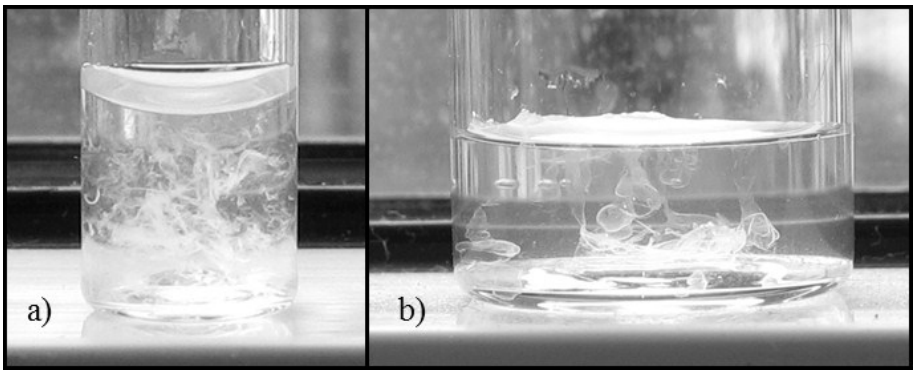


Fig. 51. Complex particles formed from cellulose sulfate (degree of substitution, DS, of 0.70, 1%, w/v) dissolved in aq. 0.5 M NaCl (a) and in water (b), and droplets of cellulose-4-[*N*-methylammonium]butyrate chloride (sample **20**, DS of 0.63, 1% in aq. 0.5 M NaCl, w/v).

NaCl, w/v). A precipitate was obtained as a resultant complex from the electrostatic interaction of the ammonium groups with the sulfate moieties. However, using aqueous solutions, smooth particles were obtained at same polymer concentration (Fig. 51b). Similar behaviour was reported in the literature for polyacrylamide complexes and it was attributed to the combination of salting-out and electric shielding effects. The salting-out effect leads to an increase of the particle mass, while at higher ionic strength, the shielding effect results at first in a swelling and then at a critical salt concentration in a dissolution of the complex particles [134].

Complex formation of hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride with aq. alginate solution

The ammonium moieties of hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride also formed a complex with the carboxyl groups in uronic acids of alginate. Aqueous solution of hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride (sample **24**, DS of 0.46, 1%, w/v) was dripped in aqueous alginate solution (2%, w/v) and well-definite spherical particles were obtained (Fig. 52a).

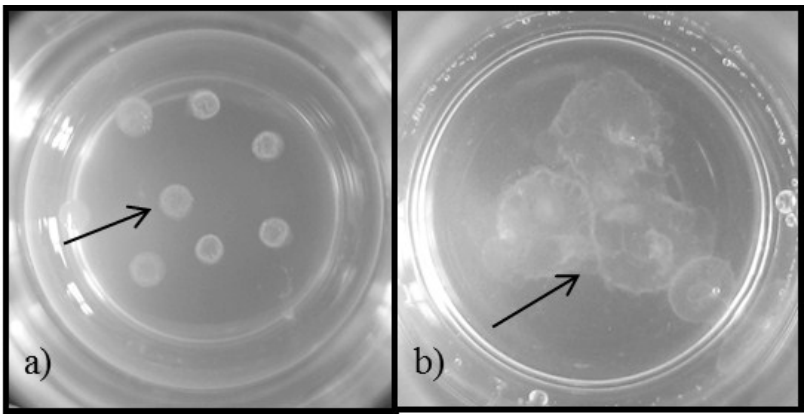


Fig. 52. Complex particles formed from aqueous alginate solution (2%, w/v) and droplets of: aqueous hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride solution (a, sample **24**, DS of 0.46, 1%, w/v) and aqueous cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride (b, sample **49**, DS of 0.75, 2%, w/v). Complexes are indicated by arrows.

According to the literature, the definite position of the complex borderline depends on the molar mass and on the concentration of the polyelectrolyte components employed [135].

Very smooth particles were formed with aqueous solution of cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride (sample **49**, DS of 0.75, 2%, w/v) dripped in aqueous alginate solution (2%, w/v, Fig. 52b). The binding of alginate to polycations as chitosan has been published in the literature [136].

Studies on the complex formation process from cellulose sulfate and PDADMAC were important and allowed the microencapsulation of biological material [133, 137]. The preliminary results showed here motivated further research about the interaction of cellulose-4-[*N*-methylammoniumbutyrate] chloride with an ultrathin cellulose support. This interaction is important for the development of biomimetic materials based on polysaccharides and it is investigated using a quartz crystal microbalance with dissipation monitoring (QCM-D) at University of Maribor [138].

3.3.3. Film formation of cellulose-4-[*N*-methylammonium]butyrate chloride onto polyethylene terephthalate film and glass surface coating

Preliminary tests were performed with cellulose-4-[*N*-methylammonium]butyrate chloride in order to verify its ability to form films. A polyethylene terephthalate film was covered with droplets of aqueous solution of sample **11** (1%, w/v) and dried in oven at 50°C. The cellulose-4-[*N*-methylammonium]butyrate chloride film (Fig. 53) was easily separated from the support due to the high flexibility of the polyethylene terephthalate film.

Coating of a glass surface was performed with droplets of aqueous solution of sample **11** (1%, w/v) and the surface was dried in oven at 50°C. Cellulose-4-[*N*-methylammonium]butyrate chloride showed good adhesion onto the glass surface.

The contact angle of a droplet of water on a neat glass surface (θc = 88.2°, Fig. 54a) and on the covered glass surface with cellulose-4-[*N*-methylammonium]butyrate chloride (θc = 58.3°, Fig. 54b) was measured in order to evaluate the hydrophobicity of the surfaces. The covered glass surface was more hydrophilic (contact angle < 90°) than the neat glass surface as expected for a coating of cationic cellulose derivative. Hydrophobic cotton fabrics covered by a thin layer of

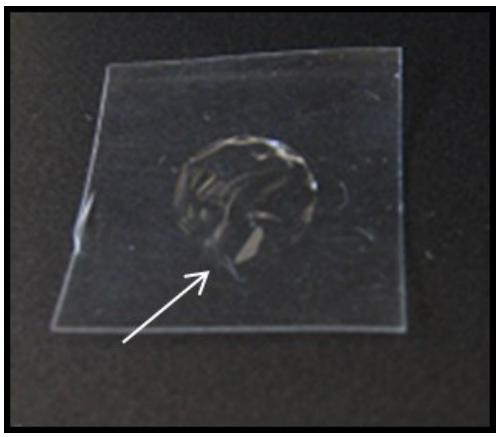


Fig. 53. Film of cellulose-4-[*N*-methylammonium]butyrate chloride (sample **11**) onto polyethylene terephthalate film, indicated by an arrow.

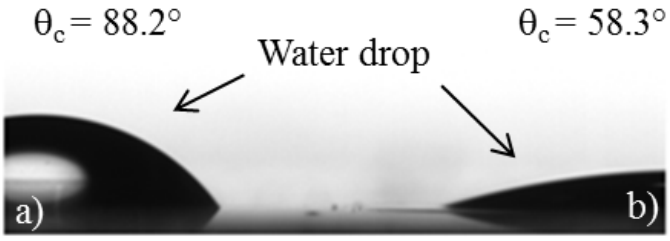


Fig. 54. Optical contact angle (θ_c) of a droplet of water on a less hydrophilic surface (a, neat glass) and on a more hydrophilic surface (b, glass surface covered with cellulose-4-[*N*-methylammonium]butyrate chloride). The contact angle was measured after the same time.

polyvinylidene fluoride (5 wt.%) had a θ_c of 132° [139]. Explanations about the wettability of solid surfaces are found in the literature [140].

A washing-test was performed with the glass surface covered by cellulose-4-[*N*-methylammonium]butyrate chloride in order to verify the stability of the coating in a water flow for 1 h (Fig. 55). After this test, the sample surface was dried and the optical contact angle with a water droplet was measured again, and it was found that the film had been completely removed by the water (θ_c = 88.2°). This experiment was repeated with cellulose-4-[*N*-methylammonium]butyrate chloride of high DP (sample **9**, DP_n of 415) and the film was also washed away. The spin coating technique has been used in the literature for the preparation of films of cellulose derivatives [141], but this technique was not efficient for cellulose-4-[*N*-methylammonium]butyrate chloride solution because no covering on a glass surface was demonstrated by contact angle measurements.

Thus, the preliminary studies performed here were important to show the ability of cellulose-4-*N*-[methylammonium]butyrate chloride to form a hydrophilic film. Further research must be carried out in order to apply this compound as a cellulosic membrane.

Fig. 55. Washing-test of glass surface covered by cellulose-4-[*N*-methylammonium]butyrate chloride immersed in a water flow-bath.

3.3.4. Antimicrobial tests of cellulose-4-[*N*-methylammonium]butyrate chlorides in aqueous solution

Cationic antimicrobial polymers as poly(4-vinyl-*N*-alkylpyridinium bromide), chitosan and other amine-containing polymers (polyethylenimines and polyhexamethylene biguanides) have been studied [142-144]. Chitosan is produced from the naturally occurring chitin and its antibacterial effect is due to its ammonium groups that interfere with bacterial metabolism by electrostatic interactions with the negatively charged cell surface of bacteria [145]. The bacteriostatic effect of the ammonium groups are still under serious discussion and different theories about it are proposed [146, 147].

Antimicrobial tests were performed in aqueous solutions of cellulose-4-[*N*-methylammonium]butyrate chlorides (samples **5**, DS of 0.31, and sample **11**, DS of 1.01) using *Staphylococcus aureus, Escherichia coli*, *Streptococcus agalactiae,* and *Candida albicans* (ASTM E 2149-01) [148]. The results of the tests performed in the Institute of Public Health of Maribor, Slovenia, are summarized in Table 8. The samples solutions were analysed at pH = 5 (highly protonated samples) and pH = 6.8 (comparable to human blood, which pH value is in the

Table 8. Results of antimicrobial test of aqueous solutions of cellulose-4-[*N*-methylammonium]butyrate chlorides, according to the ASTM E 2149-01[148].

Sample[a]	Sample conditions		Reduction of micro-organisms (%)[b]			
	Concentration (%, w/v)	pH	*Staphylococcus aureus*	*Escherichia coli*	*Streptococcus agalactiae*	*Candida albicans*
Sample **5** (DS 0.31)	0.01	5	7 (-)	23 (-)	0 (-)	22 (-)
	0.01	6.8	34 (-)	9 (-)	96 (+)	28 (-)
Sample **11** (DS 1.01)	0.01	5	15 (-)	8 (-)	0 (-)	93 (+)
	0.01	6.8	0 (-)	19 (-)	96 (+)	32 (-)
	0.03	6.8	68 (-)	66 (-)	78 (+)	98 (+)

[a] DS, degree of substitution; [b] (+) reduction of more than 75% of colonies, efficient; (-) reduction of less than 75% of colonies, inefficient.

range from 6.8 to 7.8).

According to Table 8, the growth of *Staphylococcus aureus* and *Escherichia coli*, which microbes are organized in 'bunch of grapes' clusters, was not inhibited (reduction of less than 75% of colonies) by the cellulose-4-[*N*-methylammonium]butyrate chloride solutions (0.01% and 0.03%, w/v) neither at pH = 5 nor at pH = 6.8. In comparison, 0.01% and 0.03% (w/v) of chitosan solution (in 1% of acetic acid) are the minimal inhibitory concentration (MIC) against colonies of *Escherichia coli* and *Staphylococcus aureus*, respectively [149]. In microbiology, minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation. A lower MIC is an indication of a stronger antimicrobial effect.

Sample **5** and **11** were effective against *Streptococcus agalactiae* colonies (prokaryotic microorganisms) only at pH = 6.8. Cells of this microbe form chains which are more exposed for interaction with polymer chains than a cluster. It suggests that the conformation of the bacteria could be related to its death. Compared to Penicillin, an efficient drug against *Streptococcus*

agalactiae colonies which presents a MIC = 0.000012% (w/v) in aqueous solution [150], the antimicrobial activity of cellulose-4-[*N*-methylammonium]butyrate chloride is negligible.

The higher substituted sample **11** (DS of 1.01) was able to reduce the growth of *Candida albicans* (fungi) at both pH values investigated. However, this antimicrobial activity is also negligible comparing with Floconazole (MIC = 0.0001% in aqueous solution, w/v) [151].

The test of cellulose-4-[*N*-methylammonium]butyrate chlorides showed poor bioactivity in comparison with commonly used biocides. However, the bioactivity of the cellulose derivatives synthesized in this work is comparable to chitosan since its MIC ranges from 0.005 to 0.1% (w/v) depending on the species of bacteria, on the molecular weight of the chitosan and on the pH of the solution [152, 153].

4. Materials and methods

4.1. Materials

The cellulose used were microcrystalline cellulose Avicel® PH 101 **1** ($[\eta]_{cuen}$ = 128 mL/g, DP_{cuen} of 156, DP_n 99, DP_w 339), spruce sulphite pulp HS 6 **2** ($[\eta]_{cuen}$ = 636 mL/g, DP_{cuen} of 912, DP_n 905, DP_w 12658) and cotton linters **3** ($[\eta]_{cuen}$ = 1174 mL/g, DP_{cuen} 1795, DP_n 1702, DP_w 15430). Dextran (Fluka) produced by *Leuconostoc mesenteroides* strain no. NRRL B-512(F) possesses a $\overline{M}_n$ of 38321 g/mol (DP_n = 236) and a $\overline{M}_w$ of 75432 g/mol (DP_w= 465, PDI = 1.97). The α-(1 → 6) linked glucose main chain contains about 5% of randomly distributed α-(1 → 3) branches. The polymers and LiCl were dried for 6 h at 105°C and 130°C, respectively, in vacuo over potassium hydroxide prior to use. Hydroxyethyl cellulose (MS of 3.66, DP_n = 508, DP_w = 1096, PDI = 2.16, donated by Dow Wolff Cellulosics) was dried for 6 h at 40°C as well.

BMIMCl (Iolitec) was freeze-dried prior to use. Poly(vinylsulfonic acid, sodium salt) in aqueous solution (PVS, 25%, w/v), technical grade, was purchased from Sigma-Aldrich and diluted with ultrapure water to 10 mM. (3-Carboxypropyl)trimethylammonium chloride was purchased from Sigma-Aldrich. All other chemicals were supplied by Fluka and used without further treatment.

A dialysis membrane (regenerated cellulose) with a molecular weight cut-off of 3500 g/mol was purchased from Spectra/Por®. The ionic exchanger Amberlite IRA-410 (Fluka, Cl^- form) was activated with a saturated aqueous solution of NaCl by stirring at RT for 2 h [154, 155]. The resin was isolated by filtration and washed once with distilled water.

Cellulose-2-(2-hydroxy-3-(trimethylammonium)propoxy)ethylether chloride, polymer JR400 Ucare®, molecular weight of ca. 500 000 g/mol, DS of 0.27 and charge density of 1000 g/mol of positive charges, was donated by Amerchol Corporation, Dow Chemical Company. Carboxymethyl cellulose sodium salt (DS of 0.95, low viscosity) and sodium alginate (E401-

4234) were purchased from Fluka and Hydralco respectively. Cellulose sulfate (DS of 0.7) was synthesized according Gericke *et al.* [133]. Polyethylene terephthalate film of 0.7 nm of thickness (ES301007) was obtained from Goodfellow, Germany.

4.2. Measurements

NMR spectra were acquired on a Bruker Advance 400 MHz spectrometer with 16 scans for ^{1}H NMR and up to 81920 scans for ^{13}C NMR at 60°C in deuterated dimethylsulfoxide (DMSO-d_6) and a sample concentration of 70 mg/mL. The molar degree of substitution (MS) of hydroxyethyl substituents was determined by ^{1}H NMR spectroscopy of the peracetylated products. The integrals of signals of AGU protons (5.5–2.9 ppm) were correlated to the integrals of signals of the acetate moieties (2.1–1.9 ppm) according equation 2.

Eq. 2.

$$MS_{HEC} = \frac{1}{4} \cdot \frac{9\left(\int a\right)}{\left[\left(\int b\right) - 7\right]}$$

a = signals of AGU protons (5.5–2.9 ppm), b = signals of acetate ester groups (2.1–1.9 ppm).

The FTIR spectra of the samples in KBr discs were recorded on a Nicolet Avatar 370 DTGS spectrometer. A Perkin Elmer Spectrum GX spectrometer with ATR acessory (16 scans at 4 cm^{-1} resolution, supplied by Specac Ltd., UK) was used to monitor the stability of ester moiety. Raman spectroscopy was performed in the same device and recorded over a range of 3500–100 cm^{-1} using an operating spectral resolution of 4 cm^{-1}, and a laser power output between 100 – 800 mW (32 scans for each sample). A Vario EL III (Elementaranalysensysteme Hanau, Germany) was used for elemental analyses and the halogen content was determined by a titration method after combustion [156]. The degree of substitution (DS) of the amino moieties was calculated from the elemental composition according to equation 3.

Eq. 3.

$$DS_{aminoestermoiety} = \frac{M_{AGU} \cdot N\%}{M_N \cdot 100 - M_{SG} \cdot N\%}$$

M_{AGU} = molar mass of anhydroglucose unit ($\overline{M}$ of 162.1 g/mol), N% = nitrogen content determined by elemental analysis, M_N = molar mass of nitrogen ($\overline{M}$ of 14 g/mol), M_{SG} = molar mass of the substituent group (4-[*N*-methylammonium]butyrate chloride, $\overline{M}$ of 136.5 g/mol). The molar mass of HEC unit (MS 3.66, $\overline{M}$ of 323 g/mol) was used to calculate the DS of hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chlorides.

The DP_{cuen} of the cellulose was determined by capillary viscometry applying Cuen as solvent according to ISO 5351/1999 [127]. The size exclusion chromatography (SEC) of amino (and ammonium) cellulose and dextran esters was measured in aq. 0.1 mol/L Na_2HPO_4 containing aq. 0.1% NaN_3 with a JASCO SEC system (degasser DG 980-50, pump PU 980, refractive index detector 930, columns Suprema 1000 and Suprema 100 from Kromatek, Great Dunmow, Essex, UK, with eluent flow rate of 1.0 mL/min). The eluent DMSO with 0.5% LiBr was used in combination with the columns Novema 3000 and Novema 300 (Polymer Standards Service, Mainz, Germany, eluent flow rate of 0.5 mL/min at 65°C) in the SEC analysis of dextran and percarbanilated celluloses (**1**, **2** and **3**). The eluent DMAc/0.21% LiCl was used in combination with the columns Gram 1000 and Gram 30 (Polymer Standards Service, Mainz, Germany, with eluent flow rate of 1.0 mL/min at 40°C) in the SEC analysis of non-soluble samples and hydroxyethyl cellulose. The follow procedure was used: a slurry of 500 mg (1.55 mmol) of dry hydroxyethyl cellulose in 10 mL DMAc was kept for 2 h at 120°C under stirring. After the slurry had been allowed to cool to 80°C, 0.7 g of anhydrous LiCl were added. The hydroxyethyl cellulose dissolved completely during stirring overnight and the solution was analysed by SEC. Pullulan- and dextran standards (for $M < 10^4$ g/mol) were used for calibration. Weight average- ($\overline{M}_w$) and number average ($\overline{M}_n$) molecular mass as well as polydispersity index (PDI) were determined and the degrees of polymerization (DP_n and DP_w) were calculated. UV-vis

spectrometer Lambda 10 (Perkin Elmer) was used to determine the content of tosyl groups in dextran esters. The relative (η/η_0) and intrinsic [η] viscosities were determined with an automatic viscometer (Lauda PVS 1/2) equipped with a dilution Ubbelohde viscometer (capillary No. Ic K = 0.03, EGV 821, 51713, Schott Instruments, Mainz, Germany) and with a micro Schott viscometer (capillary No. Ic K = 0.03, EGV 924, 53113, Schott Instruments, Mainz, Germany) in a thermostated water bath (Lauda E 200, Lauda-Königshofen, Germany) at 20°C. For determination of intrinsic viscosity, an automatic burette (Metrohm Dosimat 765, Filderstadt, Germany) was used to dilute the solutions automatically. An intial concentration of 4 mg/mL of hydroxyethyl cellulose (or sample **24** or polymer JR400) in water and in aqueous $NaNO_3$ (0.1 M) was chosen for the experiments. To determine the [η] values, the extrapolation of reduced viscosity to zero concentration was performed in a concentration range up to 3 mg/mL in order to minimize the influence of intermolecular interactions [157]. The [η] values were obtained by linear regression. The rheological characterization of cellulose-4-[*N*-methylammonium]butyrate chloride, hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride (2%, w/v, in water and in aq. 0.1 M NaCl) was carried out with a Haake MARS rheometer equipped with a cone-plate geometry (35 mm radius, cone-angle of 1°). Shear rates varied from 0.001 to 1000 s^{-1} at 20°C. Goniometer (OCA-35 Optical Contact Angle System, DataPhysics) was used to measure the water contact angle.

4.3. Synthesis

4.3.1. Synthesis of cellulose-4-[*N*-methylammonium]butyrate chloride

Esterification of cellulose with NMP/TosCl in NMP/LiCl [91]

A slurry of 1.0 g (6.2 mmol) dry cellulose **1** in 100 mL NMP was kept for 2 h at 120°C under stirring. After the slurry had been allowed to cool to 80°C, 9.0 g of anhydrous LiCl were added. The cellulose dissolved completely during stirring overnight without further heating yielding an optically clear solution. Pyridine (2.7 mL, 34 mmol, 5.5 mol/mol anhydroglucose unit, AGU) was added to the cellulose solution at room temperature followed by stirring for 10 min. After addition of 6.47 g (34 mmol, 5.5 mol/mol AGU) of TosCl, the mixture was allowed to react for 5 h at room temperature under stirring and 10 mL of water was added dropwise. Stirring was continued for 30 min. The polymer was isolated by precipitation with 800 mL acetone/ethanol (1:1, v/v). The solid was filtered off, washed five times with acetone and dried in vacuo at 40°C. The sample was dissolved in 40 mL of distilled water and dialyzed for 5 d against 1 L of distilled water. The water was exchanged 3 times per day. Ionic exchanger (20 g) was added to the polymer solution and the mixture was stirred overnight. Finally, the sample was isolated by filtration and freeze-dried.

Yield: 1.06 g (70.2%, sample **6**)

DS = 0.62 (calculated from nitrogen content)

Elemental analysis (%): calculated for DS of 0.62, C 44.41, H 6.58, N 3.52, Cl 8.91; found C 41.74, H 6.91, N 3.52, Cl 9.86

SEC: $\overline{M}_n$ = 15870 g/mol (DP_n = 65), $\overline{M}_w$ = 55514 g/mol (DP_w = 226), PDI = 3.50

IR (KBr): 3423 ν(OH), 2965 ν(CH_2), 1735 ν(C=O), 1282 ν(C–O–C), 1068 ν(C–O–C) cm^{-1}

^{13}C NMR (δ, DMSO-d_6): 171.5 (C-7, C=O), 102.8 (C-1, CH), 79.9 (C-4, CH), 74.7–73.3 (C-2, 3, 5, CH), 63.8 (C-$6_{substituted}$, CH_2), 60.9 (C-6, CH_2), 48.1 (C-10, C-N), 32.8 (C-11, CH_3), 31.1 (C-8, CH_2), 21.3 (C-9, CH_2) ppm

Esterification of cellulose with NMP/TosCl in BMIMCl and co-solvent [91]

A mixture of 0.5 g (3.1 mmol) of dry cellulose **1** and 4.5 g of molten BMIMCl was stirred for 12 h at 90°C to facilitate complete dissolution. NMP (10.0 mL) was added to the clear solution at 90°C under stirring. TosCl (1.18 g, 6.2 mmol, 2 mol/mol AGU) and NMP (0.6 mL, 6.2 mmol, 2 mol/mol AGU) were added after the slurry had been allowed to cool to room temperature. The homogeneous reaction mixture was stirred for 2 h at room temperature before 0.5 mL water were added dropwise. Stirring was continued for 30 min. The polymer was isolated by precipitation with 800 mL of acetone/ethanol (1:1, v/v). The solid was filtered off, washed five times with acetone and dried in vacuo at 40°C. In addition, the sample was dissolved in 20 mL of distilled water and dialyzed for 5 d against 1 L of distilled water. Ionic exchanger (20 g) was added to the polymer solution and the mixture was stirred overnight. Finally, the sample was isolated by filtration and freeze-dried.

Yield: 0.50 g (79.5%, sample **22**)

DS = 0.31 (calculated from nitrogen content)

Elemental analysis (%): calculated for DS of 0.31, C 44.43, H 6.42, N 2.13, Cl 5.39; found C 43.62, H 6.75, N 2.13, Cl 5.94

SEC: $\overline{M}_n$ = 13712 g/mol (DP_n = 67), $\overline{M}_w$ = 41514 g/mol (DP_w = 203), PDI = 3.02

IR (KBr): 3425 ν(OH), 2921 ν(CH_2), 1733 ν(C=O), 1263 ν(C–O–C), 1067 ν(C–O–C) cm^{-1}

^{13}C NMR (δ, DMSO-d_6): 172.3 (C-7, C=O), 103.2 (C-1, CH), 80.5 (C-4, CH), 75.5–70.7 (C-2, 3, 5, CH), 63.8 (C-$6_{substituted}$, CH_2), 60.9 (C-6, CH_2), 48.1 (C-10, C-N), 32.9 (C-11, CH_3), 31.0 (C-8, CH_2), 21.3 (C-9, CH_2) ppm

4.3.2. Synthesis of hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride

Esterification of hydroxyethyl cellulose with NMP/TosCl in NMP/LiCl

A slurry of 5.0 g (15 mmol) dry hydroxyethyl cellulose in 200 mL NMP (2.5%, w/v) was kept for 2 h at 120°C under stirring. The cellulose dissolved completely after stirring overnight without further heating to yield an optically clear solution. After the slurry had been allowed to cool to room temperature, pyridine (6.3 mL, 77 mmol, 5 mol/mol AGU) was added to the hydroxyethyl cellulose solution followed by stirring for 10 min. Then, 14.6 g (77 mmol, 5 mol/mol AGU) TosCl were added. The mixture was allowed to react for 5 h whilst stirring at room temperature before 40 mL of water were added dropwise. Stirring was continued for 30 min. The polymer was isolated by precipitation with 800 mL isopropanol. The solid was filtered off, washed five times with acetone and dried in vacuo at 40°C. In addition, the sample was dissolved in 500 mL of distilled water and dialyzed for 5 d against 3 L of distilled water. Ionic exchange resin (70 g) was added to the polymer solution and the mixture was stirred overnight. Finally, the sample was isolated by centrifugation and freeze-dried.

Yield: 3.80 g (76%, sample **24**)

DS = 0.46 (calculated from nitrogen content)

Elemental analysis (%): C 44.63, H 7.14, N 1.66, Cl 6.91

SEC: $\overline{M}_n$ = 137700 g/mol (DP_n = 357), $\overline{M}_w$ = 1084100 g/mol (DP_w = 2813), PDI = 1.95

IR (KBr): 3438 ν(OH), 2922 ν(CH_2), 1736 ν(C=O) cm^{-1}

^{13}C NMR (δ, DMSO-d_6): 172.6 (C-9, C=O), 102.5 (C-1, CH), 80.0 (C-4, CH), 74.7–71.8 (C-2, 3, 5, CH), 72.7 (C-8, CH_2), 70.3 (C-7, CH_2), 63.8 (C-$6_{substituted}$, CH_2), 60.8 (C-6, CH_2), 48.1 (C-12, C-N), 32.9 (C-13, CH_3), 31.0 (C-10, CH_2), 21.3 (C-11, CH_2) ppm

Esterification of hydroxyethyl cellulose with NMP/TosCl in BMIMCl and co-solvent

A mixture of 0.5 g (1.5 mmol) of dry hydroxyethyl cellulose and 6.3 g of molten BMIMCl was stirred for 12 h at 90°C to facilitate complete dissolution. NMP (10.0 mL) was added to the clear solution whilst stirring at 90°C. TosCl (1.18 g, 6 mmol, 4 mol/mol AGU) was added after the slurry had been allowed to cool to 40°C. The homogeneous reaction mixture was stirred for 5 h at 40°C before 5 mL of water was added dropwise. Stirring was continued for 30 min. The polymer was isolated by precipitation with 800 mL of isopropanol. The solid was filtered off, washed five times with acetone and dried in vacuo at 40°C. In addition, the sample was dissolved in 200 mL of distilled water and dialyzed for 5 d against 1 L of distilled water. Ion exchange resin (20 g) was added to the polymer solution and the mixture was stirred overnight. Finally, the sample was isolated by centrifugation and freeze-dried.

Yield: 0.37 g (67%, sample **33**)

DS = 0.28 (calculated from nitrogen content)

Elemental analysis (%): C 43.76, H 6.42, N 1.09, Cl 12.23

IR (KBr): 3442 ν(OH), 2878 ν(CH_2), 1739 ν(C=O) cm^{-1}

^{13}C NMR (δ, DMSO-d_6): 172.4 (C-9, C=O), 102.9 (C-1, CH), 82.3 (C-4, CH), 74.7–70.3 (C-2, 3, 5, CH), 72.8 (C-8, CH_2), 70.3 (C-7, CH_2), 63.8 (C-$6_{substituted}$, CH_2), 60.9 (C-6, CH_2), 48.2 (C-12, C-N), 43.9 (C-$6_{deoxychloro\ moiety,}$ CH_2-Cl), 33.0 (C-13, CH_3), 30.9 (C-10, CH_2), 21.3 (C-11, CH_2) ppm

4.3.3. Synthesis of dextran-4-[*N*-methylammonium]butyrate chloride

Esterification of dextran with NMP/TosCl in NMP/LiCl

A slurry of 2.0 g (12.3 mmol) dry dextran in 60 mL NMP (3%, w/v) was kept for 2 h at 120°C whilst stirring. After the slurry had been allowed to cool to 80°C, 0.6 g of anhydrous LiCl were added. The dextran dissolved completely during stirring overnight without further heating to yield an optically clear solution. Pyridine (68 mmol, 5.5 mol/mol AGU) was added to the dextran solution at room temperature followed by stirring for 10 min. Then, 12.9 g (68 mmol, 5.5 mol/mol AGU) of TosCl was added. The mixture was stirred at room temperature for 16 h before 10 mL of water was added dropwise. Stirring was continued for 30 min. The polymer was isolated by precipitation in 800 mL of acetone/ethanol (1:1, v/v), washed 4 times with 600 mL of acetone/ethanol (1:1, v/v) and dried in vacuo at 40°C. The sample was re-precipitated from 40 mL of water in 600 mL acetone. The solid was filtered off, washed five times with acetone and dried in vacuo at 40°C. In addition, the sample was dissolved in 40 mL distilled water and dialyzed for 5 d against 500 mL of distilled water. Ion exchange resin (10 g) was added to the polymer solution and the mixture was stirred overnight. Finally, the sample was isolated by filtration and freeze-dried.

Yield: 2.47 g (86%, sample **39**)

DS = 0.52 (calculated from nitrogen content)

Elemental analysis (%): calculated for DS of 0.52, C 44.42, H 6.54, N 3.14, Cl 7.94; found C 44.90, H 6.89, S 1.36, N 3.14, Cl 6.38

SEC: $\overline{M}_n$ = 33356 g/mol (DP_n = 143), $\overline{M}_w$ = 71089 g/mol (DP_w = 305), PDI = 2.13

IR (KBr): 3386 ν(OH), 2929 ν(CH_2), 1735 ν(C=O) cm^{-1}

^{13}C NMR (δ, DMSO-d_6): 172.2 (C-7, C=O), 128.5 (C-12, CH), 126.0 (C-13, CH) 98.8 (C-1, CH), 95.9 (C-1', CH), 76.6–68.5 (C-2, 3, 4, 5, CH), 67.1 (C-6, CH_2), 48.1 (C-10, C-N), 33.4 (C-11, CH_3), 30.6 (C-8, CH_2), 21.5 (C-9, CH_2) ppm

Esterification of dextran with NMP/TosCl in BMIMCl

A mixture of 1 g (6.2 mmol) of dry dextran and 9 g of molten BMIMCl was stirred for 12 h at 90°C to facilitate complete dissolution. TosCl (12.3 mmol, 2 mol/mol AGU) and NMP (12.3 mmol, 2 mol/mol AGU) were added after the slurry had been allowed to cool to room temperature. The homogeneous reaction mixture was stirred for 5 h at room temperature before 10 mL of water was added dropwise. Stirring was continued for 30 min. The polymer was isolated by precipitation in 800 mL of acetone/ethanol (1:1, v/v). The solid was filtered off, washed five times with 600 mL of acetone and dried in vacuo at 40°C. In addition, the sample was dissolved in 40 mL of distilled water and dialyzed for 5 d against 500 mL of distilled water. Ion exchange resin (10 g) was added to the polymer solution and the mixture was stirred overnight. Finally, the sample was isolated by filtration and freeze-dried.

Yield: 0.74 g (68%, sample **42**)

DS = 0.12 (calculated from nitrogen content)

Elemental analysis (%): calculated for DS of 0.12, C 44.45, H 6.28, N 0.91, Cl 2.33; found C 39.91, H 7.05, N 0.91, Cl 2.16

SEC: $\overline{M}_n$ = 24890 g/mol (DP_n = 140), $\overline{M}_w$ = 45544 g/mol (DP_w = 256), PDI = 1.83

IR (KBr): 3421 ν(OH), 2928 ν(CH_2), 1730 ν(C=O) cm^{-1}

^{13}C NMR (δ, DMSO-d_6): 172.3 (C-7, C=O), 100.7 (C-1, CH), 76.6–68.6 (C-2, 3, 4, 5, CH), 67.0 (C-6, CH_2), 48.3 (C-10, C-N), 33.1 (C-11, CH_3), 31.0 (C-8, CH_2), 21.5 (C-9, CH_2) ppm

4.3.4. Synthesis of cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride

Esterification of cellulose with (3-carboxypropyl)trimethylammonium chloride/CDI in DMAc/LiCl and DMSO

Dry cellulose **1** (1.0 g, 6.2 mmol) and 30 mL of DMAc were stirred at 120°C for 2 h. After the slurry had been allowed to cool to 80°C, 1.8 g of anhydrous LiCl was added. The reaction mixture was allowed to cool to room temperature whilst stirring, and the cellulose was observed to dissolve completely. (3-Carboxypropyl)trimethylammonium chloride (1.1 g, 6.2 mmol) was added to a solution of 1 g (6.2 mmol) of CDI in 30 mL of DMSO to obtain the corresponding imidazolide. The mixture was stirred for 1 h at 70°C and was then added to the cellulose solution in DMA/LiCl. The reaction mixture was stirred for 20 h at 70°C, poured into 600 mL of acetone/ethanol (60:40, v/v), and the polymer was collected by filtration. After washing with 400 mL of acetone/ethanol (60:40) for five times, the polymer was dissolved in water and reprecipitated in acetone. The polymer was subsequently dried at 40°C in vacuo.

Yield: 1.2 g (85.3%, sample **44**)

DS = 0.36 (calculated from nitrogen content)

Elemental analysis (%): calculated for DS of 0.36, C 46.31, H 6.81, N 2.28, Cl 5.77; found C 42.72, H 7.31, N 2.28, Cl 5.61

SEC: $\overline{M}_n$ = 13848 g/mol (DP_n = 63), $\overline{M}_w$ = 66040 g/mol (DP_w = 299), PDI = 4.77

IR (KBr): 3333 ν(OH), 2903 ν(CH_2), 1740 ν(C=O) cm^{-1}

^{13}C NMR (δ, DMSO-d_6): 172.6 (C-7, C=O), 103.2 (C-1, CH), 80.4 (C-4, CH), 75.6 – 72.5 (C-2, 3, 5, CH), 65.3 (C-$6_{substituted}$, CH_2), 64.0 (C-6, CH_2), 61.0 (C-10, C-N), 53.1 (C-11, CH_3), 31.7 (C-8, CH_2), 18.5 (C-9, CH_2) ppm

Esterification of cellulose with (3-carboxypropyl)trimethylammonium chloride/CDI in BMIMCl

Dry cellulose **1** (0.5 g, 3.2 mmol) and 4.5 g of molten BMIMCl (10%, w/w) were mixed at 80°C and kept for 12 h to assure complete dissolution. A solution of 0.5 g CDI (3.2 mmol, 1 mol/mol AGU) and 0.56 g (3-carboxypropyl)trimethylammonium chloride (3.2 mmol, 1 mol/mol AGU) in 2.5 g of molten BMIMCl was stirred for 1 h at 80°C and subsequently added to the cellulose solution. The mixture was allowed to react for 5 h at 80°C. The homogeneous reaction mixture was cooled to room temperature and poured into 600 mL of acetone/ethanol (60:40, v/v). The polymer was collected by filtration. After washing five times with 400 mL of acetone/ethanol (60:40, v/v), the polymer was dissolved in water and reprecipitated in acetone. Finally the polymer was dried at 40°C in vacuo.

Yield: 0.49 g (87.0%, sample **55**)

DS = 0.16 (calculated from nitrogen content)

Elemental analysis (%): calculated for DS of 0.16, C 45.45, H 6.51, N 1.21, Cl 3.06; found C 43.10, H 7.12, N 1.21, Cl 3.85

SEC: $\overline{M}_n$ = 12108 g/mol (DP_n = 64), $\overline{M}_w$ = 67660 g/mol (DP_w = 359), PDI = 5.59

IR (KBr): 3426 ν(OH), 2919 ν(CH_2), 1738 ν(C=O) cm^{-1}

^{13}C NMR (δ, D_2O): 174.4 (C-7, C=O), 102.9 (C-1, CH), 79.2 (C-4, CH), 75.5 – 73.7 (C-2, 3, 5, CH), 66.1 (C-10, C-N), 63.7 ($C\text{-}6_{substituted}$, CH_2), 60.8 (C-6, CH_2), 53.7 (C-11, CH_3), 30.7 (C-8, CH_2), 18.5 (C-9, CH_2) ppm

4.3.5. Percarbanilation of cellulose [4]

In a typical reaction, 300 mg of cellulose **1** was stirred with 10 mL of pyridine for 35 min at room temperature. Then 6 mL of phenylisocyanate was added to the solution. The mixture was

stirred at 80ºC for 24 h. After cooling to room temperature, 3 mL of methanol was added in order to react with any residual phenylisocyanate. The reaction mixture was poured into 300 mL of methanol/water solution (80/20, v/v). The polymer was filtered off on a sintered glass funnel and washed three times using 100 mL of methanol each time. Finally, the sample was dried for 10 h at 40°C in vacuo.

Yield: 254 mg (84.7%)

DS = 2.80 (calculated from nitrogen content)

Elemental analysis (%): calculated for DS of 2.80, C 62.06, H 4.84, N 7.92; found C 61.50, H 4.74, N 7.92

IR (KBr): 3420 ν(OH), 2919 ν(CH_2), 1637 ν(C=O), 1319 ν(C–O–C), 1057 ν(C– O–C) cm^{-1}

SEC: $\overline{M}_n$ = 20090 g/mol (DP_n = 99), $\overline{M}_w$ = 95468 g/mol (DP_w = 339), PDI = 4.75

4.3.6. Peracetylation of hydroxyethyl cellulose [92, 158]

Dry hydroxyethyl cellulose (200 mg) was stirred with 20 mL of DMAc for 1 h at 120°C. Then, 1.8 g of dry LiCl was added after the slurry had been allowed to cool to 80°C. 2 mL of acetyl chloride was added in the solution. The mixture was stirred continuously for 40 h at 80ºC. The reaction was allowed to cool to room temperature. Then, the reaction mixture was poured into 500 mL of water. The polymer was filtered-off, freeze-dried and subsequently stored in a vacuo oven for 5 h at 60°C.

Yield: 194 mg (97%)

MS_{HEC} = 3.66 (determined by ^{1}H NMR spectroscopy)

IR (KBr): no ν(OH), 2963 ν(CH_2), 1743 ν(C=O), 1096 ν(CO) cm^{-1}

^{1}H NMR (δ, $CDCl_3$): 5.1 – 2.9 (H_{AGU}, CH_2- and CH-hydrogens of hydroxyalkyl substituents), 2.1 – 1.9 (CH_3-hydrogens of acetyl groups), 1.2 – 1.0 (CH_3-hydrogens of hydroxyalkyl substituents) ppm

4.3.7. Synthesis of 4-[methylammonium]butyric acid chloride (MABA) [159, 160]

N-methyl-2-pyrrolidone (20.0 g, 201.8 mmol) and concentrated hydrochloric acid (11.0 g, 302.6 mmol) were stirred at reflux (around 150°C) for 24 h. The progress of the reaction was checked by thin layer chromatography (TLC) on silica gel with methanol/concentrated ammonia solution (3:1); the spots were detected with iodine vapour. After the slurry has been allowed to cool to room temperature, the volatiles (excess acid) were removed by rotary evaporation. The product was washed five times with acetone (100 mL) and recrystallized from *N,N'*-dimethylformamide. The crystals were washed with 50 mL of acetone and dried for 48 h at 100 °C in vacuo.

Yield: 12 g (60%); m.p. 122 - 125 °C (found), 120 - 121°C (according to reference [160])

Elemental analysis: calculated C 39.10, H 7.87, N 9.12, Cl 23.08; found C 39.25, H 8.19, N 9.05, Cl 23.30

IR (KBr): 3430 ν(OH), 2994 ν(CH_2), 1734 ν(C=O), 1183 ν(C-O-C) cm^{-1}

^{1}H NMR (DMSO-d_6): 1.80 (quin, 2 H), 2.32 (t, 2 H), 2.46 (s, 3 H), 2.83 (t, 2 H), 9.07 (s, 2 H) ppm

4.4. Determination of tosyl groups in dextran esters

An aqueous solution of dextran-4-[*N*-methylammonium]butyrate chloride (0.0179%, w/v, sample **39**) was prepared and measured by UV-vis. Dilutions of 1 mL in 10 mL (3.3 times), 2 mL in 10 mL (5 times), and 3 mL in 10 mL (10 times) of the sample solution in water were used.

The final result was calculated as an average of these three measurements. Methyl 6-O-tosyl-α-D-glucopyranoside was applied as standard to quantify the amount of tosyl moieties. The calibration curve was prepared with absorbance values at λ_{max} = 227 nm in the range of concentration between 1.95 x 10^{-5} mol/L and 1.30 x 10^{-4} mol/L (absorbance between 0.2468 nm and 1.6336 nm).

4.5. Polyelectrolyte titrations [106, 113]

Polyelectrolyte titrations were carried out in an aqueous medium at different pH values (pH = 2, 3, 4, 6, 7, 8, and 9), adjusted by addition of NaOH (0.1 M) and HCl (0.1 M), and measured using a glass electrode (Mettler Toledo DG 111-SC). The measurements were performed within 7 min in an aqueous solution of (1%, w/v) cellulose-4-[*N*-methylammonium]butyrate chloride (samples **5** and **11**, see Table 1) and of cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride (sample **44**, see Table 4). For a typical measurement at pH = 2, 0.1 mL of dissolved cellulose-4-[*N*-methylammonium]butyrate chloride (1% in water, w/v) was pipetted into a titration vessel and 1 mL of 0.1 mM indicator *O*-toluidine blue was added. The sample was diluted to a volume of 40 mL with an aqueous solution of pH = 2 that was adjusted with a known volume of HCl. A Mettler Toledo DL 53 titrator with a 10 mL burette was used for addition of PVS (10 mM) in increments of 100 μL every 3–10 s. The endpoint was detected by a Mettler Toledo Phototrode DP660 that emits light at λ = 660 nm into the solution and this light is reflected back to the photo sensor from the solution. The millivolt signal is determined by the amount of light reflected from the solution (1000 mV = 100% transmittance) [161]. At the endpoint, the absorption of light by the indicator is decreased on the solution (change in color: from blue to rose), increasing the millivolt signal [162]. The experiments were performed at room temperature. The titration results were expressed as an average value of three measurements.

4.6. Potentiometric titrations [106, 113]

Potentiometric titrations were carried out at ionic strength of aq. 0.1 M adjusted with KCl at room temperature. A two-burette instrument (Mettler Toledo) equipped with a combined glass electrode (potencial Ag/AgCl reference electrode, Mettler Toledo DG 117) was used. Aqueous solutions of 0.1 M HCl/0.1 M KCl and 0.1 M KOH/0.1 M KCl were used. The solutions were prepared with ultrapure water having very low carbonate content ($\leq 10^{-4}$ M), which was achieved through boiling and subsequent cooling under nitrogen atmosphere. The measurements were performed in aq. solution (0.3%, w/v) of cellulose-4-[*N*-methylammonium]butyrate chloride (samples **5** and **11**, Table 1) and of cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride (sample **44**, Table 4). 10 mL of aq. cellulose-4-[*N*-methylammonium]butyrate chloride solution (0.3%, w/v)/0.1 M KCl was pipetted into a titration vessel and diluted with 20 mL of aq. 0.1 M KCl. The sample was titrated in forward and backward runs between pH = 2.5 and pH = 11.5, with a previous addition of 0.5 mL of hydrochloric acid (0.1 M). The titrant was added at varied intervals of 0.001–0.25 mL. The stability criterion during the analysis after each titrant addition was set at *dE/dt* = 0.1 mV/50 s, and 50 s was the minimum time for reaching equilibrium conditions between two additions of the titrant. A fast titration (*dE/dt* = 0.1 mV/10 s) of cellulose-4-[*N*-methylammonium]butyrate chloride was only used to determine its pK. A blank HCl-KOH titration was performed under the same conditions, in order to eliminate any error due to the presence of water contaminants, such as dissolved CO_2. The titrant volume was normalized to the mass of the titrated cellulose derivatives and expressed as mol of charge per unit mass (in mmol/g) vs. pH curve. The charging isotherms, in terms of mmol of charged ionic species as function of pH, were calculated from the potentiometric titration curves as difference between the hydrogen-acidities of the analysed sample and the blank. The experiments were performed at room temperature.

Calculation of the charging isotherms [104, 106]

The ionic species present in the titrated system are the strong K^+ and Cl^-, the pH-determining H^+ and OH^-, and potentially conjugated species of the analyte (ammonium group-containing cellulose ester), A_k^n, where *n* represents the charge number and *k* is an enumerator. The net ionic charge *Q*, due to the presence of A_k^n can be calculated from the electroneutrality condition as shown in Eq. 4.

Eq. 4.

$$Q(\mathrm{pH}) = FV_t \sum_k n\left[A_k^n\right] = FV_t\left(\left[Cl^-\right] - \left[K^+\right] + \left[OH^-\right] - \left[H^+\right]\right)$$

The square brackets denote the concentration in mol/L, V_t is the total volume and *F* is the Faraday constant. The concentrations of potassium ($[K^+]$) and chlorine ions ($[Cl^-]$) are known from the added volumes and from the concentrations of the burette solutions, while the concentration difference of hydrogen and hydroxyl ions ($[OH^-]$ - $[H^+]$) is determined by the pH. For the blank titration, only K^+, Cl^-, H^+ and OH^- are present, thus $Q = 0$ for any measured pH value. This permits replacing the term ($[OH^-]$ - $[H^+]$) in Eq. 4 with ($[K^+]_{blank}$ - $[Cl^-]_{blank}$). Therefore, the excess charge due to the presence of the analyte can be calculated as shown in Eq. 5.

Eq. 5.

$$Q_{analyte}(\mathrm{pH}) = FV_t\left(\left[Cl^-\right] - \left[K^+\right] + \left[K^+\right]_{blank} - \left[Cl^-\right]_{blank}\right)$$

$[K^+]_{blank}$ and $[Cl^-]_{blank}$ denote the blank concentrations of potassium and chloride ions, respectively. The values of ($[K^+]_{blank}$ - $[Cl^-]_{blank}$) at the pH values measured for the analyte system can be obtained by interpolating the blank titration curves. Alternatively, the difference ($[OH^-]$ - $[H^+]$) could be calculated directly from the measured pH and introduced into Eq. 4 in order to calculate $Q_{analyte}$. However, the former approach is recommended because it permits the elimination of errors due to the presence of water contaminants like carbonic acid from any dissolved CO_2, as well as the utilization of semiempiric formulas for the activity coefficients

(Davies, for instance). The $Q_{analyte}$(pH) curves are referred to as the 'charging isotherms'. From the charging isotherm of forward titration, the dissociation constant (pK) was calculated by nonlinear least squares fitting.

4.7. Hydrolysis study

Total organic carbon and nitrogen content of cellulose-4-[*N*-methylammonium]butyrate chloride

30 mL of 0.1% (w/v) solution of cellulose-4-[*N*-methylammonium]butyrate chloride (sample **11**) was potentiometrically titrated from pH 2.5 to pH 7 and a 5 ml aliquot was collected as aliquot A at pH = 7. The same sample solution was then further titrated, within the range of 7 < pH < 11.5, which resulted in the formation of a irreversible precipitate. In addition, it was back-titrated from pH 11.5 to pH 7, and an aliquot B (5 mL) was collected at pH = 7. The precipitate was removed from the aliquot B by centrifugation in a Centric 332A (Tehtnica) centrifuge. Aliquots A and B (without precipitate) were subjected to TOC/TN measurements which were performed in a Multi N/C 2100 S (Analytik Jena AG) instrument. The results were expressed as mmol of carbon (or nitrogen) per gram of sample.

Influence of storage time on amino group content by polyelectrolyte titration

To study the influence of storage time and sample stability, acidic and neutral aq. solutions of cellulose-4-[*N*-methylammonium]butyrate chloride (sample **11**, 1%, w/v, pH 3, 6 and 7) were prepared and the pH was adjusted with HCl (0.1M). A weakly alkaline aq. solution (1%, w/v) was prepared by addition of some drops of buffer solutions at pH 10 ($Na_2B_4O_7$/NaOH) and pH 7 (KH_2PO_4/Na_2HPO_4), to acquire pH 8. The samples were analysed with regard to the amino groups' content by polyelectrolyte titration as described above. The samples were analysed

immediately after the preparation of the initial solutions and then at the following time intervals: 30 min, 1 h, 2 h, 6 h, 12 h, 24 h, 48 h, 1, 2, 3, and 4 weeks.

Stability of ester moiety by ATR-FTIR- and Raman spectroscopy

Films were prepared with the same solutions used for the study of the influence of storage time. In a petri dish, 1 mL of the solution (sample **11**, 1%, w/v, at adjusted pH) was blow dried with nitrogen gas and analysed by ATR-FTIR. Spectra (16 scans at 4 cm^{-1} resolution) were recorded at room temperature and rationed to the appropriate background spectrum. A Perkin Elmer Spectrum GX spectrometer with ATR accessory (supplied by Specac Ltd., UK) was used.

The Raman spectra were recorded over a range of 3500-100 cm^{-1} using an operating spectral resolution of 4 cm^{-1} and a laser power output between 100-800 mW. 32 scans were performed for each sample on a Perkin Elmer Spectrum GX spectrometer. A 1064 nm near-IR laser was applied as a light source for the excitation of Raman scattering. Spectra were obtained from cellulose-4-[*N*-methylammonium]butyrate chloride sample **11**, using different sample preparations. A spectrum was generated by measuring the sample in the solid state, shortly after its synthesis. Subsequently, cellulose-4-[*N*-methylammonium]butyrate chloride sample **11** was dissolved in ultrapure water (1%, w/v) and the pH of the solution was adjusted to pH 12, forming a precipitate that was freeze-dried. The freeze-dried precipitate was analysed by Raman spectroscopy and originated the second spectrum.

4.8. Influence of storage time by capillary viscosimetry

Cellulose-4-[*N*-methylammonium]butyrate chloride (sample **11**) was dissolved in aqueous 0.1% trifluoroacetic acid/0.05 M NaCl (pH = 2), water/0.05% NaN_3 (pH = 7), 0.1 M Na_2HPO_4/0.05% NaN_3 (pH = 9) to a concentration of 4 mg/mL (0.4%, w/v) and kept for 63 days at room

temperature (22°C). The aqueous saline solutions without samples were stored under the same conditions and used as a blank. Aliquots were taken every week and subjected to capillary viscometry to determine their relative viscosity.

4.9. Preparation of polyelectrolyte complexes

Cellulose-4-[*N*-methylammonium]butyrate chloride (sample **5**, **11** and **20**), hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride (sample **24**) and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride (sample **49**) were dissolved in water or in aq. 0.5 M NaCl and used as polycation in the complex formation. Alginate, carboxymethyl cellulose and cellulose sulfate were used as the polyanion component. For a typical experiment, an aqueous solutions of cellulose-4-[*N*-methylammonium]butyrate chloride (1%, w/v, sample **5**, DS of 0.31) was added to an aqueous solution of carboxymethyl cellulose (DS of 0.95) dropwise using a syringe (Omnikan 20, G27x1/2’’; 0.4 x 13 mm).

4.10. Film formation of cellulose-4-[*N*-methylammonium]butyrate chloride

Cellulose-4-[*N*-methylammonium]butyrate chloride (sample **11**) was dissolved in water (1%, w/v). With a pasteur pipette, 1 mL of cellulose solution was deposited onto the surface of a flat bottom cylindrical glass vial and onto the surface of a polyethylene terephthalate film of 0.7 nm of thickness (ES301007, Goodfellow supplier, Germany). The films were then dried in oven for 4 h at 50°C. The films were stored in a desiccator until measurement. A water droplet (3 μL) was deposited upon the film surface and the contact angle was measured by goniometer (OCA-35 Contact Angle System, DataPhysics).

Washing-test of cellulosic/glass film

A beaker filled with 250 mL of distilled water under magnetic stirring was used as water flow-bath. The cellulosic/glass film was placed on a wire mesh and immersed in water flow for 1 h. After this time, the sample was dried in an oven for 4 h at 50°C and the contact angle of the surface with a droplet of water (3 µL) was measured by goniometry.

4.11. Antimicrobial test

The antimicrobial activity of cellulose-4-[*N*-methylammonium]butyrate chloride (sample **5** and **11**) was evaluated by modified ASTM E2149-01 [148] under dynamic contact conditions. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Streptococcus agalactiae* and *Candida albicans* were used as test organisms. An incubated test culture in a nutrient broth was diluted using a sterilized 0.3 mM phosphate buffer (KH_2PO_4, pH = 6.8 and pH = 5) in order to give a final concentration of 1.5–3.0 x 10^5 colony forming units (CFU)/mL. This solution was used as a working bacterial dilution. 1 mL of aqueous solutions of cellulose-4-[*N*-methylammonium]butyrate chloride (samples **5** and **11**, 0.5% or 1.5%, w/v) was transferred to a 250 mL Erlenmeyer flask containing 50 mL of the working bacterial dilution (final concentration of cellulose-4-[*N*-methylammonium]butyrate chloride of 0.01% and 0.03%, w/v). All flasks were loosely capped, placed in the incubator, and shaken for 1 h at 37 °C and 120 rpm using a Wrist Action incubator shaker. After a series of dilutions using the buffer solutions, 1 mL of the diluted solution was plated in nutrient agar. The inoculated plates were incubated at 37 °C for 24 h and the surviving cells counted. The average values of the duplicates were converted to CFU/mL in the flasks, by multiplying with the dilution factor. The antimicrobial activity was expressed as

percent of reduction of organisms after contact with the test specimen, compared to the number of bacterial cells surviving after contact with the control.

5. Summary

The synthesis of novel water-soluble cationic amino esters based on cellulose and dextran under homogeneous conditions was the main motivation of the present work. Products were synthesized via the ring-opening reaction of *N*-methyl-2-pyrrolidone (NMP) in the presence of *p*-toluenesulfonyl chloride: cellulose-4-[*N*-methylammonium]butyrate chloride, hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride and dextran-4-[*N*-methylammonium]butyrate chloride. The structures were characterized in detail by elemental analysis, FTIR- and NMR-spectroscopy. Some polymer degradation occurred in the conversions as confirmed by size-exclusion chromatography (SEC).

Cellulose-4-[*N*-methylammonium]butyrate chlorides with a degree of substitution (DS) in the range from 0.31 to 1.04, and with a degree of polymerization (DP_n) in the range from 33 to 415 were synthesized under mild reaction conditions (at room temperature, up to 16 h reaction time). Deoxychloro moieties were undesirable moieties formed by nucleophilic substitution and were identified in samples prepared at reaction temperatures above 40°C. NMP/LiCl and 1-butyl-3-methylimidazolium chloride (BMIMCl) were used as solvents. Products with higher DS were obtained in reactions performed in NMP/LiCl using higher molar ratio of reagents than in the ionic liquid. However, reactions performed in BMIMCl were advantageous since they allowed the use of a polymer concentration of 10% (w/w), they generate products with a higher DP, and furthermore, lower molar ratios of reagents were required to accomplish the reactions.

The reaction using hydroxyethyl cellulose (HEC) was more difficult: HEC was well soluble in NMP without addition of LiCl, however the reaction mixture was very viscous. Nucleophilic substitution on the methylene groups formed undesirable deoxychloro moieties, however some products without such moieties were able to be synthesized and characterized. Novel hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chlorides, which contain an ether and an

ester moiety in the same structure, were obtained with a DS range from 0.28 to 0.98, and it is believed that they may prove to be an alternative to commercially available polymers.

Dextran was converted in a comparable way in order to obtain cationically modified derivatives. Comparing to cellulose, dextran was less reactive due to the fact that it has no primary hydroxyl group at position 6 available for modification. Products with a DS range from 0.12 to 0.72 and a narrow DP_n range from 114 to 159 were obtained under similar reaction conditions.

Cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride was obtained by conversion of cellulose with (3-carboxypropyl)trimethyl ammonium chloride activated with *N,N'*-carbonyl diimidazole. *N,N*-Dimethylacetamide/LiCl and BMIMCl were used as a reaction media. High reaction temperatures (up to 100°C) and reaction times (up to 20 h) were necessary to yield products with DS in the range from 0.17 to 0.75 and DP in the range from 18 to 124. No side reactions were detected during the synthetic procedure. The samples were characterized by elemental analysis, SEC, FTIR- and NMR-spectroscopy.

The positive charges on cellulose-4-[*N*-methylammonium]butyrate chloride and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride in aqueous solutions at various pH values (from pH = 2 to pH = 9) were quantified by polyelectrolyte titration. Potentiometric titration was used to titrate the amino moiety and a difference between the titration curves from pH = 2 to pH = 10 (forward) and from pH = 10 to pH = 2 (backward) was found, indicating structural instability at alkaline pH value. The combination of the titration results with Raman- and FTIR-spectra of the sample solutions showed that:

- Hydrolysis of the ester moiety occurs at aqueous solutions of pH ≥ 8 and was already observable after 2 h.

- Cellulose-4-[*N*-methylammonium]butyrate chloride is positively charged and its aqueous solution is stable for at least 1 month at acidic (pH = 3, pH =6) and neutral (pH= 7) pH values.

The investigation of properties of cellulose-4-[*N*-methylammonium]butyrate chloride, hydroxyethyl cellulose-4-[*N*-methylammonium]butyrate chloride and cellulose-4-[*N,N,N*-trimethylammonium]butyrate chloride showed that:

- The samples increase the viscosity of water up to 17 times (from 0.001 Pa.s to 0.017 Pa.s). This property depends on the DS and on the DP of the dissolved cellulose derivative. The viscosity is stable for at least 2 months in solutions at acidic (pH = 2) and neutral (pH = 7) pH values.

- Polyelectrolyte complexes with sodium alginate, carboxymethyl cellulose and cellulose sulfate can be formed.

- Films onto polyethylene terephthalate can be formed and glass surfaces coated with cellulose-4-[*N*-methylammonium]butyrate chloride possess hydrophilicity.

- Cellulose-4-[*N*-methylammonium]butyrate chloride has no antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, and poor activity against *Streptococcus agalactiae* and *Candida albicans*.

6. Zusammenfassung

Ziel der Arbeit war die Synthese neuer wasserlöslicher aminogruppenhaltiger Cellulose- und Dextrancarbonsäureester unter homogenen Reaktionsbedingungen. Die Ringöffnungsreaktion von *N*-Methyl-2-pyrrolidon (NMP) in Gegenwart von *p*-Toluensulfonsäurechlorid ergab die Chloride von Cellulose-4-[*N*-methylammonium]butyrat, Hydroxyethylcellulose-4-[*N*-methylammonium]butyrat und Dextran-4-[*N*-methylammonium]butyrat. Die Struktur der Produkte wurde mittels Elementaranalyse sowie FTIR- und NMR-Spektroskopie detailliert aufgeklärt. Die Untersuchungen mittels Größenausschlusschromatographie lassen einen Polymerabbau durch die chemische Modifizierung erkennen.

Es wurden das Chlorid von Cellulose-4-[*N*-methylammonium]butyrat mit durchschnittlichen Substitutionsgraden (DS) von 0,31 bis 1,04 und durchschnittlichen Polymerisationsgraden (DP) von 33 bis 415 unter milden Reaktionsbedingungen (Raumtemperatur, Reaktionszeit bis 16 h) synthetisiert. Bei Reaktionstemperaturen ab 40 °C traten unerwünschte Nebenreaktionen auf, wie die Bildung von 6-Deoxy-6-chlorogruppen durch nukleophile Substitution. NMP/LiCl und 1-Butyl-3-methylimidazoliumchlorid (BMIMCl) wurden als Lösungsmittel für die Polysaccharide eingesetzt. Umsetzungen in NMP/LiCl ergaben auf Grund des größeren Reagenzüberschusses Produkte mit höherem DS als Reaktionen in der ionischen Flüssigkeit. Demgegenüber erlaubt BMIMCl eine höhere Polymerkonzentration (10 %) im Reaktionsmedium. Die Produkte weisen einen höheren DP_n auf und kleinere Reagenzmengen sind erforderlich.

Die Umsetzung von Hydroxyethylcellulose (HEC) hat sich als schwieriger erwiesen: HEC ist in NMP ohne Zusatz von LiCl löslich. Allerdings sind die so erhaltenen Lösungen sehr viskos. Durch nukleophile Substitutionsreaktionen an den Methylengruppen wurden meist unerwünschte 6-Deoxy-6-chlorofunktionen erhalten. Es konnten aber auch Produkte ohne Nebenstrukturen synthetisiert, was aus der detaillierten Charakterisierung hervorgeht. Hydroxyethylcellulose-4-[*N*-methylammonium]butyrat-chloride mit DS-Werten von 0.28 bis 0.98 wurden erhalten, die

sowohl Ether- als auch Esterstrukturen enthalten und eine Alternative zu kommerziell erhältlichen Polymeren sein können.

Dextran wurde analog wie die Cellulose zu kationischen Derivaten umgesetzt. Im Vergleich zu Cellulose ist Dextran weniger reaktiv, da keine primären Hydroxylgruppen an Position 6 vorhanden sind. Produkte mit DS-Werten zwischen 0,12 und 0,72 sowie in einem engen DP-Bereich von 114 bis 159 wurden synthetisiert.

Cellulose-4-[*N,N,N*-trimethylammonium]butyrat-chlorid konnte durch die Umsetzung von Cellulose mit (3-Carboxypropyl)trimethylammoniumchlorid unter Aktivierung mit *N,N'*-Carbonyldiimidazol hergestellt werden. *N,N*-Dimethylacetamid/LiCl und BMIMCl dienten als Reaktionsmedien. Hohe Reaktionstemperaturen (bis 100°C) und Reaktionszeiten (bis 20 h) waren notwendig, um Produkte mit DS-Werten zwischen 0.17 und 0.75 zu synthetisieren. Die DP-Werten lagen im Bereich von 18 bis 124. Nebenreaktionen wurden nicht beobachtet. Die Proben wurden mittels Elementaranalyse, GPC sowie FTIR-und NMR-Spektroskopie untersucht. Die positiven Ladungen an Cellulose-4-[*N*-methylammonium]butyrat-chloriden und Cellulose-4-[*N,N,N*-trimethylammonium]butyrat-chloriden in wässriger Lösung wurden bei pH-Werten von 2 bis 9 mittels Polyelektrolyttitration quantifiziert. Die Aminogruppen wurden mittels potentiometrischer Titration bestimmt. Aus den Titrationskurven von pH 2 bis 10 (vorwärts) und von pH 10 bis 2 (rückwärts) geht hervor, dass die Struktur unter alkalischen Bedingungen instabil ist. Die Kombination beider Titrationsergebnisse zeigte, dass die Hydrolyse der Estergruppen bei pH ≥8 und bereits nach 2 h erfolgt. Cellulose-4-[*N*-methylammonium]butyrat-chlorid ist positiv geladen. Die wässrige Lösung ist bei pH 3, 6 und 7 für wenigstens einen Monat lagerstabil.

Die Untersuchung der Eigenschaften der Chloride von Cellulose-4-[*N*-methylammonium]butyrat, Hydroxyethylcellulose-4-[*N*-methylammonium]butyrat und Cellulose-4-[*N,N,N*-trimethylammonium]butyrat hat ergeben, dass die Proben die Viskosität von Wasser ungefähr auf das 17-fache (von 0,001 Pa s auf 0,017 Pa s) erhöhen. Diese Eigenschaft

hängt sowohl vom DS als auch vom DP des gelösten Cellulosederivates ab. Die Viskosität ist bei Lagerung im sauren (pH 3) und neutralen Medium (pH 7) für wenigstens 2 Monate stabil. Es können Polyelektrolytkomplexe mit Natriumalginat, Carboxymethylcellulose sowie Cellulosesulfat gebildet werden. Die Derivate bilden hydrophile Filme auf Polyethylenterephthalat. Cellulose-4-[*N*-methylammonium]butyrat-chlorid weist im Gegensatz zu vielen kationischen Polymeren keine biologische Aktivität gegenüber *Staphylococcus aureus* und *Escherichia coli* und nur eine geringe Wirkung gegenüber *Streptococcus agalctiae* und *Candida albicans* auf.

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8. Publication list

- Mohan, T., Zarth, C. S. P., Zemljič, Doliška, A., Kargl, R., Grießerd, T., Spirk, S., Heinze, T., Stana-Kleinschek, K., Interactions of a cationic cellulose derivative with an ultrathin cellulose support. *Carbohydrate Polymers* 2012, manuscript accepted.

- Zarth, C. S. P., Zemljič, L. F., Čakara, D., Bračič, M., Pfeifer, A., Stana-Kleinschek, K., Heinze, T., Charging behavior and stability of the novel amino group-containing cellulose ester cellulose-4-[*N*-methylamino]butyrate hydrochloride. *Macromolecular Chemistry & Physics* 2012, in press, DOI = 10.1002/ macp.201200057.

- Zarth, C. S. P., Koschella, A., Pfeifer, A., Dorn, S., Heinze, T., Synthesis and characterization of novel amino cellulose esters. *Cellulose* 2011, *18*, 1315-1325.

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11. Eidesstattliche Selbstständigkeitserklärung

Ich erkläre, dass ich die vorliegende Arbeit selbständig und nur unter Verwendung der angegebenen Hilfsmittel, persönlichen Mitteilungen und Quellen angefertigt habe.

Jena, den 02.08.2012 ----------

(Cíntia Salomão Pinto Zarth)