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Aminocelluloses – Polymers with Fascinating Properties and Application Potential

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1.1 Introduction

Cellulose is a linear D-glucan containing β-1 → 4 linkages and is the world's most abundant natural polymer with an estimated annual global production of about 1.5×10^{12} tons and, hence, a very important renewable and sustainable resource [1]. Although unmodified cellulose is used largely as paper, board, and fibers, there is huge space to design novel and advanced products based on cellulose by its chemical modification. In particular, esters and ethers of cellulose are most important [1, 2].

Due to their low-cost production, biodegradability, and low-toxicity cationized polysaccharides are promising in fields of effluent treatment, papermaking, and food, cosmetic, pharmaceutical, petroleum, and textile industries, as well as in analytical chemistry and molecular biology [3]. In particular, cationic cellulose derivatives gain increasing interest in different scientific and industrial fields, e.g. as flocculation agents [4], being an alternative to toxic polyacrylamide. In Germany, the disposal of sludge treated with polyacrylamides has been forbidden in areas under cultivation since 2014 [5].

Considering the recent literature, the huge amount of publications was summarized in reviews about cationic synthetic polyelectrolytes [6] as well as cationized polysaccharides (amino and ammonium hydroxypropyl ethers) [3]. However, in this chapter, the authors will not review the cationic ethers; the overview refers to cationic esters, 6-deoxy-6-amino cellulose derivatives, and amino carbamates of cellulose. In spite of the industrial applications that are usually associated with cationic polymers, a variety of advanced polymer coatings providing sophisticated features, e.g. biosensors or immuno assays, will be presented.

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1.2 Amino-/ammonium Group Containing Cellulose Esters

1.2.1 (3-Carboxypropyl)trimethylammonium Chloride Esters of Cellulose

An efficient approach to cationic cellulose derivatives is the esterification of the hydroxyl groups with cationic carboxylic acids. Activated carboxylic acids such as acyl chlorides or acid anhydrides are not appropriate due to their limited solubility, availability, and the formation of acidic by-products. However, the esterification applying imidazolides obtained from the corresponding carboxylic acid and *N*,*N*-carbonyldiimidazole (CDI) is a mild and efficient synthesis strategy [2].

To synthesize cationic cellulose esters (3-carboxypropyl)trimethylammonium chloride was activated with CDI in dimethylsulfoxide (DMSO) separately and allowed to react with cellulose dissolved in *N*,*N*-dimethylacetamide (DMA)/LiCl [7]. Thus, a product with a degree of substitution (DS) of 0.75 was accessible that could be characterized by ^{13}C NMR spectroscopy (Figure 1.1).

Cellulose (3-carboxypropyl)trimethylammonium chloride esters adsorbed on cellulose films may trigger the protein adsorption, which is a key parameter in the design of advanced materials for a variety of technological fields [8]. The protein affinity to the surface can be controlled by the charge density and solubility, adjusted by the pH value, the concentration of protein and the DS of the tailored cationic cellulose derivative. To understand the influence of the cationic cellulose ester on the protein affinity, the interaction capacity with fluorescence-labeled bovine serum albumin (BSA) at different concentrations and pH values was carried out (Figure 1.2). The adsorbed material was quantified applying QCM-D (quartz crystal microbalance with dissipation monitoring, wet mass) and MP-SPR (multi-parameter surface plasmon resonance, dry mass). Thus, the amount of coupled water in the layer could be evaluated by a combination of QCM-D and surface plasmon resonance (SPR) data. According to these studies the interaction decreases in order of

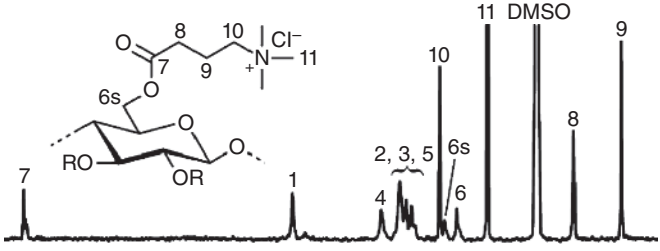

Figure 1.1 ^{13}C NMR spectrum of cellulose (3-carboxypropyl)trimethylammonium chloride ester in DMSO-d_6. *Source*: Vega et al. 2013 [7]. Reproduced with permission of American Chemical Society.

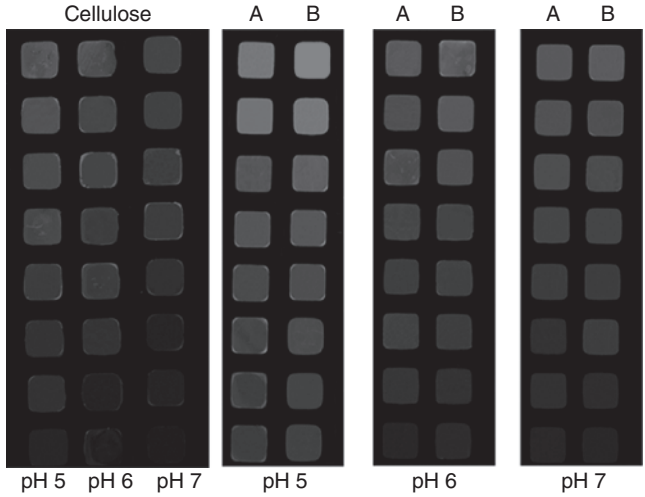

Figure 1.2 Cyclic olefin polymer slides equipped with cellulose and cellulose (3-carboxypropyl)trimethylammonium chloride ester incubated with different concentrations of labeled BSA (1000, 500, 100, 10, 1, 0.1, 0.01, and 0.001 μg mL^{-1}) at different pH values. A) low DS; B) high DS [8]. Reproduced with permission of American Chemical Society. *(See insert for color representation of this figure.)*

pH 5 > pH 6 > pH 7 and $DS_{high} > DS_{low}$, respectively. The adsorption of BSA may be adjusted over a range from 0.6 to 3.9 mg m^{-2} (dry mass). This approach is suitable to utilize BSA as blocking agent on the surface and achieve selective functionalization of cellulosic surfaces by functional proteins (e.g. antibodies).

Another application of (3-carboxypropyl)trimethylammonium chloride esters of cellulose is the surface modification of pulp fibers in order to preserve the inherent bulk properties (e.g. low density, mechanical strength) and to improve the properties of the fiber surface (e.g. wetting behavior, bacteriostatic activity) [7]. In recent studies, polyelectrolyte complexes (PECs) were prepared applying the cationic cellulose ester and anionic xylan derivatives, which were subsequently adsorbed to wood fibers. The adsorption process was studied using polyelectrolyte titration and elemental analysis. The fiber surfaces modified were characterized by X-ray spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS). The measurements evidence the interaction between the pulp fibers and the PECs and provide useful information about the adsorption process.

In addition to monofunctional cationic cellulose (3-carboxypropyl)trimethylammonium chloride esters, multifunctional photoactive derivatives provide advanced features in context with the design of smart materials. However, sufficient DS values are required to give a pronounced photochemical response and water solubility. Therefore, different cellulose 2-[(4-methyl-2-oxo-2H-chromen-7-yl)oxy]acetates were prepared applying CDI and the corresponding

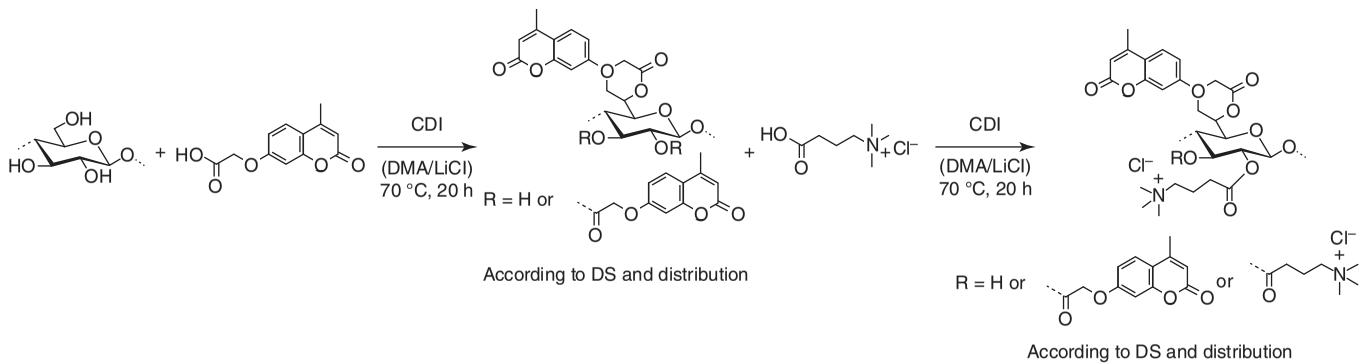

Figure 1.3 Synthesis scheme of cellulose 2-[(4-methyl-2-oxo-2H-chromen-7-yl)oxy]acetates and cellulose 2-[(4-methyl-2-oxo-2H-chromen-7-yl)oxy]acetate [4-(*N*,*N*,*N*-trimethylamonium) chloride] butyrates by *in situ* activation of 2-[(4-methyl-2-oxo-2H-chromen-7-yl)oxy]acetic acid and (3-carboxypropyl)trimethylammonium chloride with *N*,*N*-carbonyldiimidazole (CDI) in *N*,*N*-dimethylacetamide/LiCl (DMA/LiCl).
Source: Wondraczek et al. 2012 [9]. Reproduced with permission of Springer Nature.

carboxylic acid in DMA/LiCl [9]. Subsequently, (3-carboxypropyl)trimethylammonium chloride activated with CDI forming the corresponding imidazolide was allowed to react with the photoactive cellulose derivative to obtain a water-soluble product (Figure 1.3). The partial DS values could be determined by a combination of UV–Vis spectroscopy and elemental analysis. The DS is in the range from 0.05 to 0.37 for the photoactive moiety and from 0.19 to 0.34 for the cationic group.

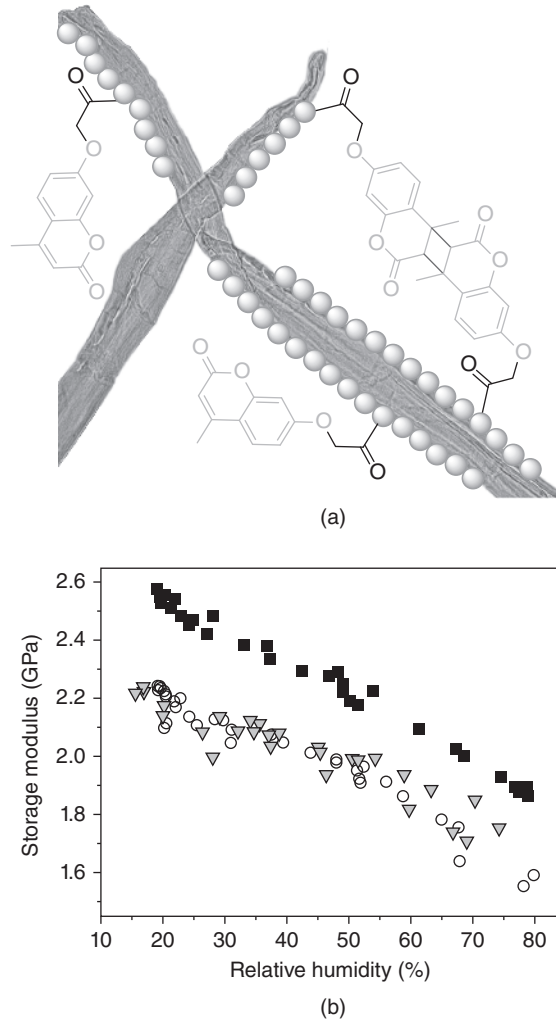

Figure 1.4 Crosslinking by [2+2] cycloaddition of the photoactive cellulose derivative on fibers (a), storage stimulus at varying relative humidity obtained by dynamic mechanical analysis (b), white/circles: unmodified fibers, light gray/triangles: fibers modified with photoactive cationic cellulose derivative, dark gray/squares: modified fibers irradiated upon sheet formation.

Multifunctional, i.e. photoactive and cationic, cellulose esters were used for the coating of pulp fibers to yield new fiber-based materials, whose properties could be triggered by an external stimulus [10]. The adsorption of the polymer onto the fiber was studied by UV–Vis spectroscopy and SPR. It turned out that electrostatic interaction is the main driving force of the adsorption. However, there is a contribution of hydrophobic interactions between the fibers and the cellulose derivatives and between the polymer chains themselves. Considering the adsorption behavior, UV–Vis measurements of the solutions applied for coating led to a mechanism according to the Freundlich model. ToF-SIMS imaging revealed evenly distributed derivatives on the fiber surfaces independent of the dosage and DS of the photoactive group. Moreover, UV irradiation of the modified fibers results in crosslinking by [2+2] cycloaddition of the photoactive moieties and both light adsorption and fluorescence behavior change (Figure 1.4). Moreover, there is an enhancement of the tensile strength and Z-directional tensile strength of the pulp fibers by 81% and 84% compared to the unmodified fiber network [11]. The stiffness of individual fibers is increased by 60%. It is supposed that this work opens new pathways for the development of smart bio-based materials being superior to classical pulp and paper.

Recently, 6-deoxy-6-azido-carboxmethyl cellulose could be synthesized [12]. Although it is possible to adsorb this anionic polymer on cellulose mediated by multivalent metal cations [13], it is much more promising to use cellulose modified with cationic moieties for this approach due to the anionic nature of pulp and cellulose surfaces in general. Thus, conversion of 6-deoxy-6-azido cellulose with carboxypropyltrimethylammonium chloride in the presence of CDI yielded 6-deoxy-6-azido cellulose-2,3-*O*-[4-(*N*,*N*,*N*-trimethyl-ammonium)]butyrate chloride (Figure 1.5) [14]. In a different approach, this multifunctional cellulose derivative could be applied for coating of fiber interfaces in aqueous media. According to this concept, the cationic cellulose derivative may adsorb to the surface anionic groups from the cellulose fiber and the azido moiety provides the covalent linkage of various functionalities via copper(I)-catalyzed azide-alkyne Huisgen cycloaddition (click chemistry) [15]. Thus, photoactive- as well as amino-group-containing fibers could be obtained applying 1-ethynylpyrene or propargylamine. It was shown that the cycloaddition between reactive fibers and alkyne groups could be carried out in aqueous medium and in organic solvents. Field emission scanning electron microscopy (FE-SEM) images revealed the preservation of the fiber structure during the preparation of photo-fibers.

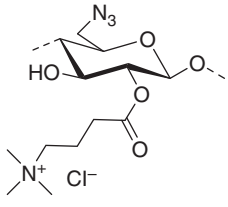

Figure 1.5 Structure of 6-deoxy-6-azido cellulose-2,3-*O*-[4-(*N*,*N*,*N*-trimethylammonium)] butyrate chloride.

1.2.2 Cellulose-4-(*N*-methylamino)butyrate (CMABC)

An alternative synthesis path to obtain cationic cellulose esters is the ring-opening of lactams in the presence of *p*-toluenesulfonic acid chloride [16]. Cellulose, dissolved in *N*-methyl-2-pyrrolidone (NMP)/LiCl, or 1-butyl-3-methylimidazolium chloride, could be transformed into the cationic biopolymer derivative applying NMP, *N*-methyl-2-piperidone, ε-caprolactam and *N*-methyl-ε-caprolactam. The lactam, e.g. NMP, forms a reactive intermediate in the presence of tosyl chloride according to the Vilsmeier–Haack reaction (Figure 1.6). Thus, a cationic cellulose ester is formed in the second step, i.e. the iminium ion reacts with the hydroxyl groups of the biopolymer and subsequent ring opening by water occurs. The products obtained possess DS values in the range from 0.24 to 1.17.

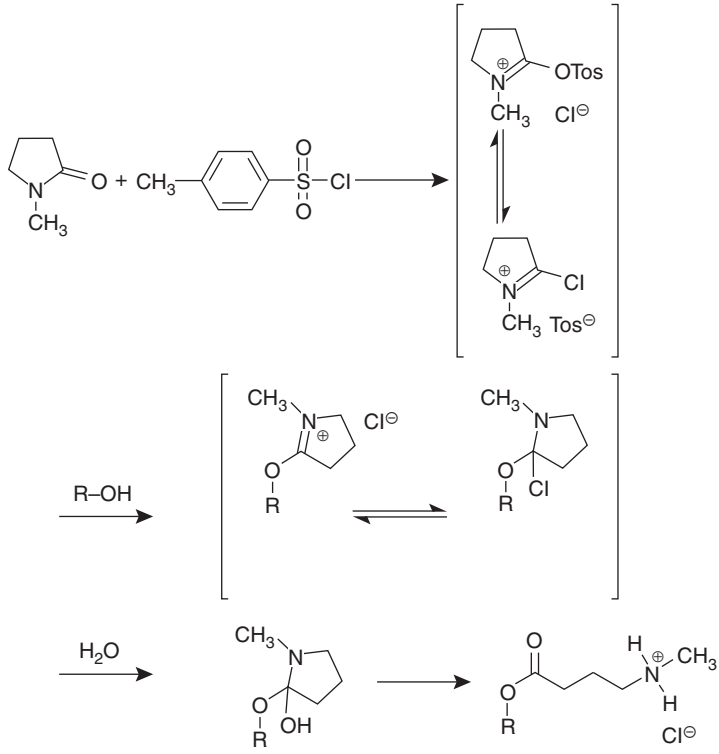

Figure 1.6 Reaction scheme of the conversion of alcohols (R–OH) with *N*-methyl-2-pyrrolidone in the presence of *p*-toluenesulfonic acid chloride.

With respect to applications of cellulose-4-(*N*-methylamino)butyrate (CMABC), the stability in aqueous solutions and the charging behavior of amino moieties was studied [17]. Samples of the cationic cellulose esters do not hydrolyze at pH values up to 7. Decomposition of the biopolymer derivatives in cellulose and carboxylate takes place at higher pH values as revealed by titration experiments, FTIR and Raman spectroscopic studies. However, the application of CMABC in fields of flocculants or thickener is promising due to the decomposition in alkaline media subsequent to its use.

As mentioned, the improvement of the properties of paper and fibers by coatings of cationic cellulose derivatives gain increasing interest. Thus, it is essential to analyze interactions of positively charged polymers with cellulose surfaces. However, monitoring of the adsorption on fibers is difficult, laborious and requires a combination of analytical techniques. An elegant way to study the adsorption behavior of CMABC in aqueous solution is the use of cellulose model thin films applying a highly sensitive surface technique such as QCM-D [18]. It turned out that at high ionic strength (25–100 mM NaCl) high adsorption is observed at pH 7 ($\Delta f = -15$ to -17 Hz), while at lower ionic strength (1–10 mM) the adsorption decreases ($\Delta f = -2$ to -12 Hz) indicated by lower absolute values of the shifts in frequency (Figure 1.7). A change in pH value from 7 to 8 caused an increased adsorption. The conformation of CMABC at low electrolyte concentration is flat-like leading to a thin layer on the cellulose substrate, which was shown by atomic force microscopy (AFM). Increasing the ionic strength, the conformation of the polymer is structured like a particle (coil). This phenomenon is associated with reduced solubility of CMABC and more material is adsorbed on the surface. The irreversibility of the adsorption process is related to interactions of cellulose and CMABC possessing structural similarities. The surface wettability increases with an increasing amount of cationic polymer on the surface. CMABC does not adsorb onto cellulose at pH values of 3 and 5. The results were validated by the determination of

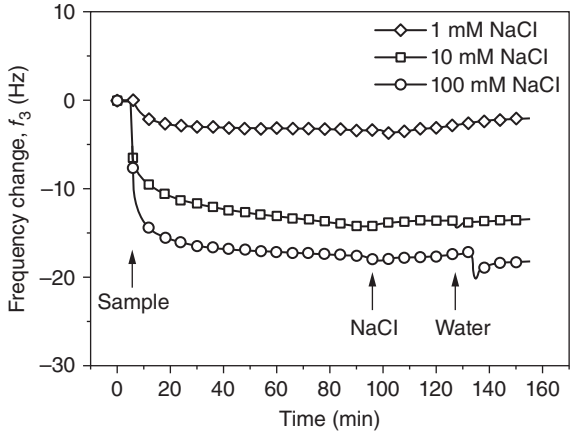

Figure 1.7 Changes in the third overtone frequency (Δf_3) for the adsorption of cellulose-4-(*N*-methylamino) butyrate onto cellulose surfaces at pH 7 depending on NaCl concentration.

the nitrogen content obtained from XPS. The amount of electrolyte incorporated into the films could be determined. The adsorption on the hydrophilic and negatively charged substrate, silicon dioxide coated quartz crystals, was not observed by QCM-D measurements. The mechanism could not be explained unambiguously up to now. However, high charge, steric hindrance induced by inter- and intramolecular hydrogen bonding and reduced affinity of CMABC to the rather rigid SiO_2 surfaces seem to be relevant parameters.

1.3 6-Deoxy-6-amino Cellulose Derivatives

Amino group containing biopolymers are of huge interest in field of functional surface coatings due to the biocompatible environment and the accessible amino groups being useful for immobilization of enzymes or antibodies (Figure 1.8). The design of soluble amino cellulose derivatives is carried out

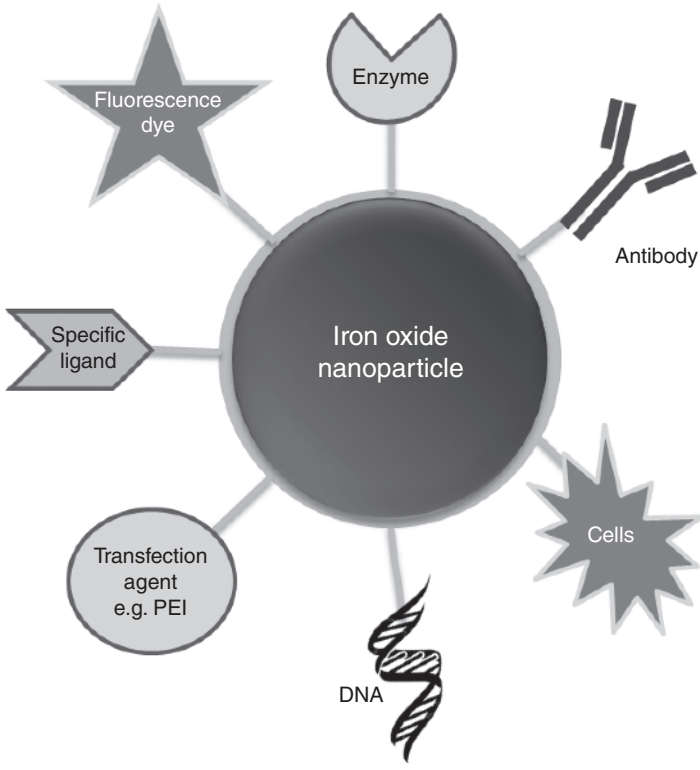

Figure 1.8 Examples of stabilized iron oxide nanoparticles modified with various multifunctional ligands and receptors. *Source*: Heinze et al. 2015 [19]. Reproduced with permission of John Wiley & Sons.

after conversion of hydroxyl groups of the polysaccharide into good leaving groups. Based on tosyl cellulose, nucleophilic displacement reactions (S_N) could be performed at primary position 6 applying di- and oligo-amines [19]. In order to prevent any crosslinking, an excess of bi- or multifunctional amine must be applied. Varying the structure of the amine, a tunable spacer length, different pK_a values and charge distributions, hydrophilic/lipophilic balance and redox chromogenic properties are provided. Moreover, modification of the secondary hydroxyl groups prior to the S_N reaction is appropriate to tailor the properties of the products. Usually, esterification of the secondary hydroxyl groups could be applied to adjust the solubility of the biopolymer derivatives.

1.3.1 Spontaneous Self-assembling of 6-Deoxy-6-amino Cellulose Derivatives

In addition to the solubility of amino cellulose in water or organic solvents depending on the detailed structure, extraordinary solution properties of the aqueous solutions were found. Reversible association products, which typically occur for proteins, could be discovered by analytical ultracentrifugation [20]. Sedimentation coefficient distributions of water-soluble 6-deoxy-6-amino celluloses were obtained from sedimentation velocity experiments in the analytical ultracentrifuge for different solute loading concentrations. The sedimentation coefficient distributions show between four and five discrete species with a stepwise increase in sedimentation coefficient. This behavior was found for the first time for polysaccharides and changes the whole conception of carbohydrate molecular interaction phenomena. Thus, it is very interesting in context with structural modeling of interfacial material surfaces with biological recognition functions at the molecular and cellular level. The partially reversible interactions of 6-deoxy-6-amino cellulose may be adapted to other biomolecules. For example, amino cellulose has a high affinity to glycoproteins and proteoglycans decorated with sugars, which provide receptor structures for an extracellular matrix (ECM). Recently, even a fully reversible self-association of tetramers was discovered for 6-deoxy-6-(ω-aminoethyl)amino cellulose. Moreover, these tetramers of amino cellulose chains associate further into supra-molecular complexes [21] (Figure 1.9).

In addition to self-association in solution, the formation of ultrathin and transparent films of amino celluloses takes place by self-assembling on planar substrates like glass, gold, and Si-wafer that could be proven by AFM. Appling 5% solutions of amino cellulose (in water or organic solvents), a nano-scaled topography is obtained by tipping, spraying, and spin coating [22–24]. The tendency of film forming decreases with decreasing basicity of the amine residue. Increasing spacer length reduces the film quality toward higher brittleness of the layers. In addition, the occurrence of gel-like particles formed in a solution of 1,4-phenylenediamine (PDA) cellulose tosylate in DMA should

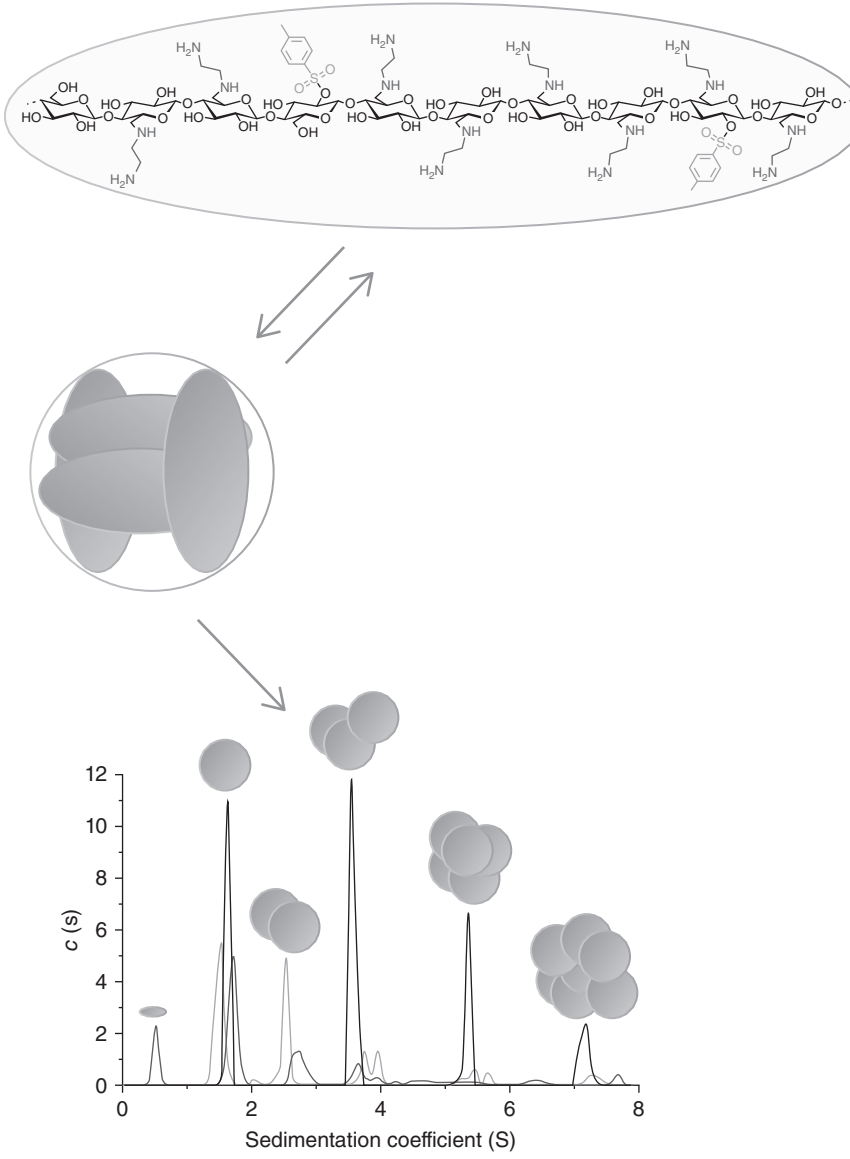

Figure 1.9 Reversible tetramerization and further higher-order association of the polysaccharide 6-deoxy-6-(ω-aminoethyl)amino cellulose.

be considered, which became visible after about one week of storage at 4 °C. Similar effects of gel formation were observed for other amino celluloses [25–27].

The aggregation behavior is influenced by the spacer structure, the basicity of the amino groups, substituents at position 2 and 3, support material and the conditions such as pH value and temperature [22, 26]. The support material possesses a very low roughness, preferably. Gold or glass coated with SiO_2 and subsequently with an organopolysiloxane are suitable surfaces for amino cellulose coatings [22, 26]. Alkylene diamine and oligoamino cellulose solutions form topographically flat films (topographies <1 nm) on SiO_2/glass, which could be explained by spontaneous adhesive interactions with the SiO_2. This counteraction to a self-aggregation of the amino cellulose chains is supposed to flatten the film surface. In contrast to SiO_2, amino functionalized polysiloxane supports led to a topographic expansion of the layer in the range of about 200 nm film thickness. However, the influence of the film support on the topography of the film is less pronounced with increasing ageing of the solution, i.e. aggregation of the amino cellulose [19].

Self-assembled monolayers (SAM)s of amino celluloses on various substrates can be obtained from dilute, aqueous polymer solutions (0.01–0.05%). The monolayer is stable against intensive rinsing with water. Moreover, organo-soluble amino cellulose forms analogous SAMs from DMA. The thickness of a SAM of ethylenediamine (EDA) cellulose is 1–2 nm as revealed by ellipsometric studies. Dimensions of amino cellulose chains and spacer length calculated by the Desktop Molecular Modeller computer program (Oxford University) agree very well with a monolayer measured by AFM (Figure 1.10).

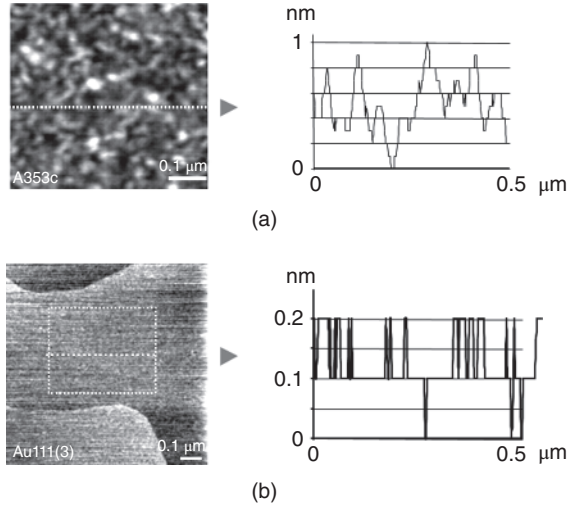

Figure 1.10 AFM topography and profile line of an EDA cellulose-SAM-modified Au/111/substrate indicating the roughness and thickness of ~1 nm (a) in comparison with an unmodified Au/111/substrate (b).

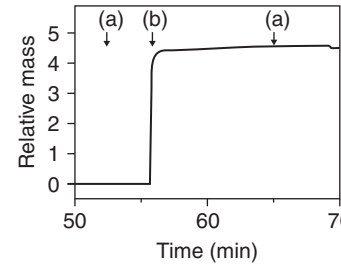

Figure 1.11 Change of relative mass versus time upon contact of a Si substrate with water (a) and a solution of TAEA cellulose (b).

The support materials, which may possess various chemical structures, are activated by piranha solution or plasma treatment. This procedure is, for example, appropriate for glass. However, other preparations of the surfaces, e.g. coatings, are required depending on the substrates. With respect to the support material, type of pretreatment and the application, the modification is carried out by mechanical shaking, shaking in an ultrasonic bath, dip coating, drop coating, or spin coating. It turned out that after approximately three minutes, adhesive mass saturation of the 6-deoxy-6-tetra(2-aminoethyl)amino cellulose carbamate (TAEA) cellulose is reached, which was revealed by kinetic measurements applying a mass-sensitive surface acoustic wave (SAW) chip in a microfluidic sensor system on Si substrates (with SiO_2 layer, Figure 1.11). Similar adhesion characteristics were, for example, found for EDA cellulose on Si and Au substrate in continuous flow operation [28].

1.3.2 Application Potential of 6-Deoxy-6-amino Cellulose Derivatives

Due to the protein-like environment of amino cellulose films, these layers are well suited to immobilize enzymes [26]. The covalent coupling with enzymes often leads to highest activities because the natural conformation of the enzyme is stabilized [29]. In addition to the microenvironment of the support material, the coupling reagent influences the enzyme activity by the hydrophilic-lipophilic balance, pH value, and ionic strength. Furthermore, the coupling to the binding site of a specific functional group defines the charges of amino acid residues. However, there are only a few systematic investigations of the influence of the coupling reagent [30, 31] and the support material [32, 33] on the properties of the immobilized enzymes. In this overview, NH_2 groups are utilized for the enzyme coupling, which is carried out by activation of the support film and subsequent connection to the biomolecule. The coupling reagents applied (Figure 1.12) are mostly homobifunctional molecules, e.g. glutardialdehyde or cyanuric chloride [22, 34, 35]. The activating agents are used in excess to avoid cross-linking, which would cause swelling of the matrix and may influence the immobilization of enzymes.

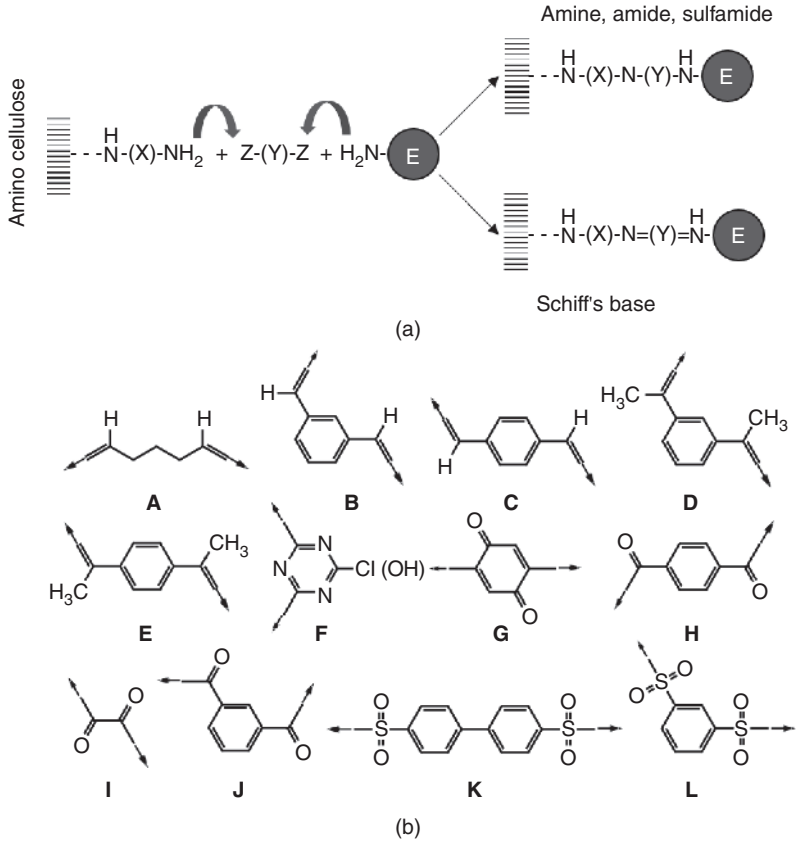

Figure 1.12 Schematic representation of enzyme immobilization on amino cellulose surfaces (a) and coupling structures (b).

Films of PDA cellulose are a support matrix with the option of coupling dyes. On the one hand, the oxidative coupling of phenols or naphthols to PDA cellulose leads to blue-stained products and the redox potentials are in the same range as biological systems (enzymes) [25]. On the other, bifunctional reagents can be applied for enzyme coupling as mentioned above. Due to the redox-chromogenic properties of PDA cellulose and a close neighborhood at molecular scale, immobilized oxidoreductases are allowed to form or consume H_2O_2 and a measureable signal can be detected.

The enzyme activity may be influenced by the interplay of support matrix, coupling structure and enzyme protein. Thus, the spacer structure has a significant influence and is responsible for the interaction between biomolecule and support matrix. Moreover, the influence of the environment, i.e. the pH-value, the distribution of electrostatic charge and the balance of hydrophilicity/

hydrophobicity at the nanostructure level might affect the parameters for enzyme activity, e.g. immobilized enzyme activity/area (mU cm^{-2}), long-term stability and enzyme-kinetic (K_M for Michaelis–Menten enzymes) [26, 36, 37].

A strong influence of the coupling structure on the values of the enzyme activity was found for glucose oxidase (GOD), lactate oxidase (LOD), and horseradish peroxidase (HRP). The unstable LOD possesses its highest enzyme activity by immobilization with benzene-1,3-dicarboxylic acid dichloride. A similar influence of the coupling and the resulting structure was found for other amino cellulose films [22, 26, 35]

It is interesting to note that the enzyme activity of immobilized GOD and HRP is preserved for a longer time compared to the dissolved enzymes. Moreover, properties of the support matrix influence the kinetic of the immobilized enzyme in comparison to the dissolved enzyme. The Michaelis–Menten enzyme kinetics (K_M value) is a measure of the enzyme/substrate affinity, which is a significant parameter for enzyme coupling. K_M limits the measureable concentration range of the analyte; the value decreases with increasing substrate and analyte-enzyme affinity [38] (Figure 1.13).

Considering the affinity of glucose to GOD, it turned out that the enzyme is incorporated efficiently into the PDA cellulose film using ascorbic acid and the affinity is increased by factor 21. The reason for this surprising effect on the K_M value seems to be the redox chromogenic PDA structure acting as an electron mediator in close molecular-geometrical neighborhood to the oxidoreductase. In contrast to films of PDA cellulose, higher K_M values were always found using NH_2 glass as the support matrix for immobilized GOD. The values were even higher than those of the native enzyme in solution. Studying other enzymes, it occurs that K_M values of immobilized HRP and LOD are slightly influenced by the coupling structure, but strongly depend on the nature of the

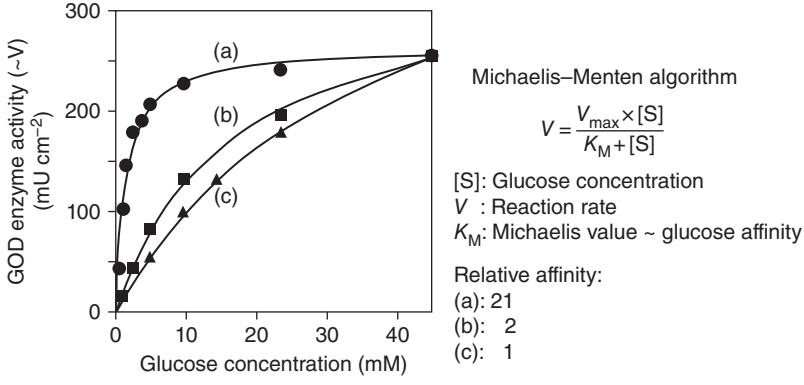

Figure 1.13 Activity of GOD according to Michaelis–Menten enzyme kinetics depending on the coupling structure to PDA cellulose films.

Table 1.1 Covalent coupling of selected oxidoreductases to PDA cellulose films by different coupling structures (Y, Figure 1.12).

		Activity (mU cm^{-2})		
Enzyme coupling	**(Y)**	**GOD**	**HRP**	**LOD**
Diazo coupling	—	194	135	220
Glutaraldehyde	**A**	187	206	100
L-Ascorbic acid	**I**	185	200	192
Benzene-1,3-disulfonic acid chloride	**L**	168	48	—
4,4′-Biphenyldisulfonic acid chloride	**K**	27	35	—
Benzene-1,3-dicarboxylic acid dichloride	**J**	34	121	286
Benzene-1,4-dicarboxylic acid chloride	**H**	60	27	131
Cyanuric chloride	**F**	33	59	119
Benzene-1,3-dialdehyde	**B**	94	58	61
Benzene-1,4-dialdehyde	**C**	56	48	21
1,3-Diacetylbenzene	**D**	51	76	123
1,4-Diacetylbenzene	**E**	70	104	52

amino functionalized support surface. However, both, the coupling reagent and the support structure are influenced by the K_M value of immobilized GOD [35] (Table 1.1).

In case of SAMs, relevant surface properties of the layer with respect to applications are the thickness, water contact angle, AFM root mean square (RMS) roughness, and the surface density of amino groups on the substrate. A concentration of NH_2 groups on the substrate modified with amino cellulose such as 0.2 nmol cm^{-2} is already relevant for application in biofunctionalization, as shown by covalent couplings of proteins such as GOD, thrombin-specific DNA, and ribonucleic acid (RNA) aptamers [23, 28, 35]. The RNA aptamer-functionalized sensor chips are able to bind thrombin in high amounts selectively without any nonspecific protein binding such as BSA or elastase. A SAW sensor chip being specific to glucose was designed by immobilization of GOD onto amino cellulose-modified gold- or SiO_2-coated surfaces applying glutardialdehyde. The support materials modified with amino cellulose could be used for a wide variety of biofunctional applications, even after storage.

Amino cellulose is well suited for microcontact printing, μCP [24]. μCP without subsequent rinsing of the substrate sample leads to structural patterns of aggregates as shown by AFM (Figure 1.14a). However, if the surface is intensively washed, the structural patterns have a thickness of about 2 nm, which is typical for composite monolayers of amino cellulose (Figure 1.14b). In order

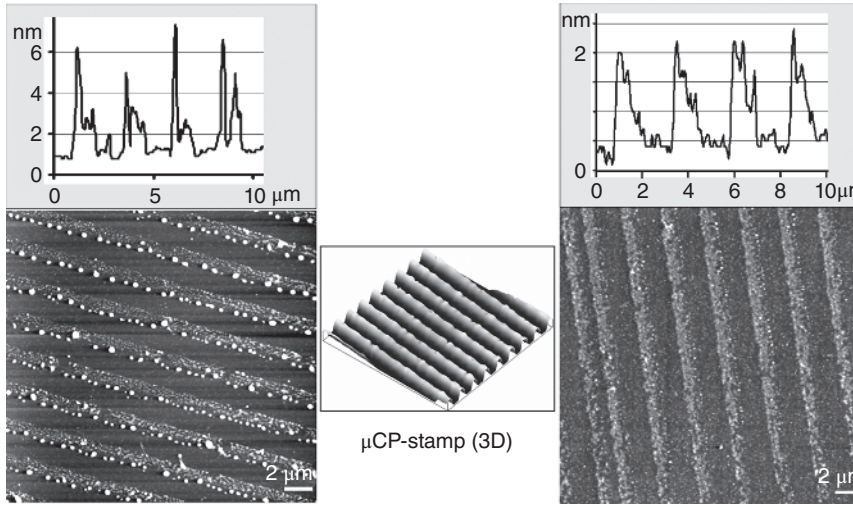

Figure 1.14 AFM images of lateral structural patterns from DPTA cellulose on $Si/SiO_2(n)$ substrate surface by µCP using 0.05% aqueous solution without rinsing (a) and subsequent washing of the substrate (b).

to enable interesting applications in the field of nanoscale biosensor or biochip design, nanostructural patterns with adhesively fixed gold nanoparticles were formed from micro contact-printed amino cellulose and monodispersive gold colloid solutions of particle sizes of 5 and 20 nm [24].

AFM tips (Si) could be modified by a 0.05% aqueous solution of TAEA cellulose. With respect to AFM experiments, it seems that amino cellulose is transferred from the tip to the gold surface as a consequence of competing adhesive interactions [24]. Si tips modified with amino cellulose are expected to be very promising in a wide range of applications, especially with regard to scanning probe lithography techniques for the lateral nanostructuring of substrate surfaces and to the studies of force spacing or force modulation in the context of a structural design of biofunctional substrate surfaces [39].

The ability of amino cellulose to form larger supramolecular structures, i.e. nanoobjects by self-assembling or spinning processes, is very promising to create advanced materials. TAEA celluloses blended with polyvinyl alcohol (PVA) were electrospun to nanofibrous webs with fiber diameters between 50 and 160 nm [40] (Figure 1.15). Contrary to chitosan-containing nanofibers, the TAEA-containing nanofiber nonwovens showed a strong reduction of *Staphylococcus aureus* growth and a significant antibacterial efficiency against the gram-negative *Klebsiella pneumoniae*.

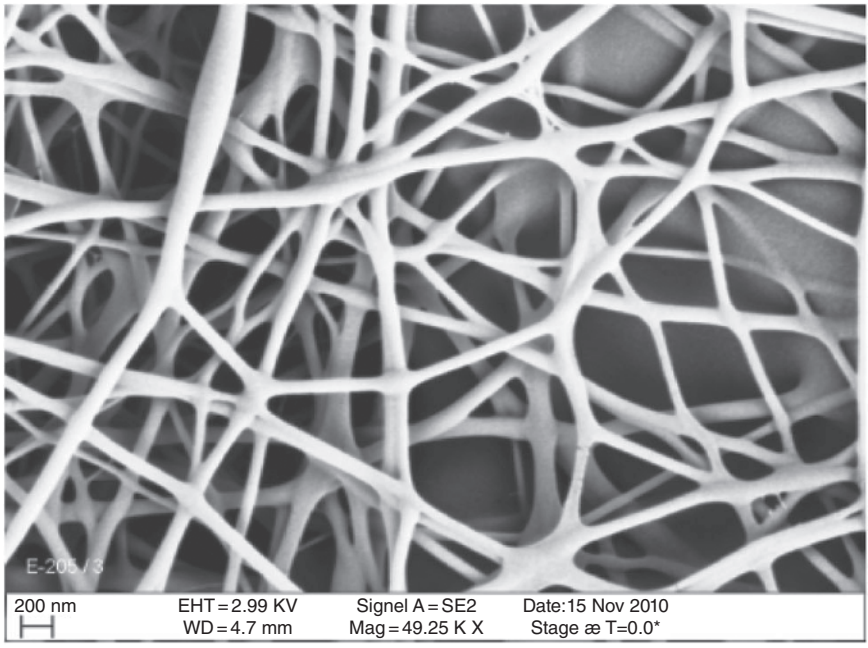

Figure 1.15 SEM picture a nanofiber web of 6-deoxy-6-tris(2-aminoethyl) amino cellulose, TAEA cellulose/polyvinyl alcohol (PVA) 1/15.

To design spherical nanoobjects, organo-soluble 6-deoxy-6-(ω-aminoalkyl) amino cellulose carbamates were allowed to self-assemble into nanoparticles with sizes from 80 to 200 nm. The particles were very stable, nontoxic, and possessed primary amino groups that were accessible for further modifications in aqueous suspension. For instance, labeling with rhodamine B isothiocyanate could be carried out without any change of size, stability, and shape as revealed by means of photo correlation spectroscopy, zeta potential measurements, scanning electron microscopy (SEM), and fluorescence spectroscopy [41]. Moreover, confocal laser scanning microscopy revealed the incorporation of these nanoparticles in human foreskin fibroblasts BJ1-hTERT and breast carcinoma MCF-7 cells without any transfection reagent.

Nanoparticles of 6-deoxy-6-(2-aminoethyl)amino (AEA) and 6-deoxy-6-{2-bis[*N*′,*N*′-(2-aminoethyl)]-aminoethyl}amino (BAEA) cellulose carbamate with a diameter of 80–120 nm exhibited significant antimicrobial activity with moderate cell compatibility [42]. On the one hand, the antibacterial activity against *S. aureus* and *K. pneumoniae* by incubation with the particles revealed a similar activity compared to a solution of the parent amino cellulose. On the other, the biocompatibility studied with HaCaT cells indicates lower toxicity of the nanoparticles compared to the solution of the amino cellulose;

an interesting phenomenon that cannot be explained satisfactorily with the present results.

Amino cellulose is not only appropriate to form biopolymer particles but also layers of amino cellulose, which can stabilize metallic nanoparticles. A promising approach from an economic and ecological point of view is the synthesis of silver nanoparticles (2–14 nm) in aqueous colloidal solutions [43]. Amino cellulose may act both as reducing agent (starting from $AgNO_3$) and as the capping material, i.e. to stabilize the colloidal system. These nanoparticles possess antibacterial effects with levels between "sufficient" and "good," and this property upholds even after storage for 18 month. Silver particles stabilized by amino cellulose can be deposited and fixed on cotton fibers or on microporous cellulose acetate filters in order to induce a significantly antibacterial effect against methicillin-resistant *Staphylococcus aureus* (MRSA). This work is interesting for medical and industrial environments since the permeability of the filters is not affected. These hybrid particles (amino cellulose/silver) and cork can be assembled by *Laccase* into an antimicrobial material with potential for water remediation [44]. The oxidation of the phenolic moieties in cork leads to reactive quinones and a subsequent reaction with nucleophilic amino groups on the particle surface was exploited to provide sustainable antibacterial activity. The biopolymer layer stabilizes the particles, acts as an interface for permanent grafting to cork and synergistically improves the antibacterial effect. The biocatalytically functionalized cork matrix reduces the growth of *Escherichia coli* and *S. aureus* efficiently during the course of five days. Long-term stability and durability of the antimicrobial effect in conditions of continuous water flow suggest the potential application of the functionalized cork matrices in constructed wetlands as an adsorbent for removal of wastewater impurities while at the same time avoiding microbial contaminations.

Magnetic nanoparticles (Fe_3O_4) could also be coated with 6-deoxy-6-(2-(bis(2-aminoethyl)aminoethyl)amino) cellulose in order to yield hybrid particles in organic media, which were characterized by a combination of light scattering, thermogravimetry, and magnetic techniques. The average diameter of the hybrid particles is about 8 nm as determined by electron microscopy and light scattering. The particles can be used as heterogeneous ligands in the ATRP of styrene [45]. Polystyrene (PS) with a near-narrow molecular weight distribution (polydispersity index, PDI < 1.3) and low Cu contents (5 ppm) was obtained. Thus, the polymeric materials possess a low copper content compared to established procedures such as activators regenerated by electron transfer – atom transfer radical polymerization (ARGET-ATRP). It was possible to separate the hybrid particles from the mixture after the reaction by an external magnetic field and reuse them in further polymerizations (Figure 1.16).

With respect to biological or medical applications, the hemocompatibility of aqueous solutions of AEA cellulose with DS values between 0.54

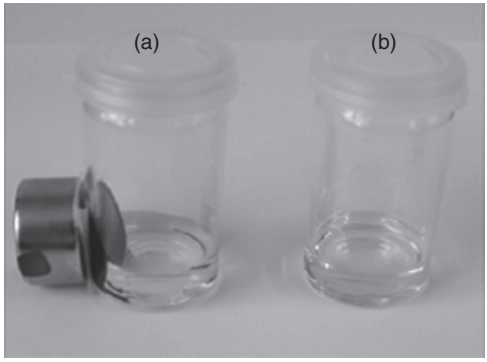

Figure 1.16 Images of polystyrene solutions after ATRP using MNP@AC50 (a) and *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA) (b) as ligands. *(See insert for color representation of this figure.)*

and 0.92 was investigated *in vivo*. AEA with a low DS of 0.54 showed the highest hemocompatibility. Therefore, AEA is suggested to be suitable for biomedical applications. However, the anticoagulative properties of AEA cellulose with high DS (0.84–0.92) could be applicable for implants to prevent thromboembolic events [46].

Organo-soluble amino cellulose derivatives could be synthesized even in a one-pot procedure by simultaneous reaction of tosyl cellulose with EDA and 4-chlorobenzylamine. The products soluble in various organic solvents form nanoparticles by nanoprecipitation with water. The polymers could be labeled with fluorescein isothiocyanate that are useful for the labeling of biomolecules in bioassays [47].

It is important to note again that the 6-deoxy-6-amino cellulose may be used to change the properties of bulk material significantly while applying only a very low amount. For instance, simple printing paper was coated with a dilute aqueous solution of 6-deoxy-6-tris(2-aminoethyl)amino cellulose (0.05 wt%) by

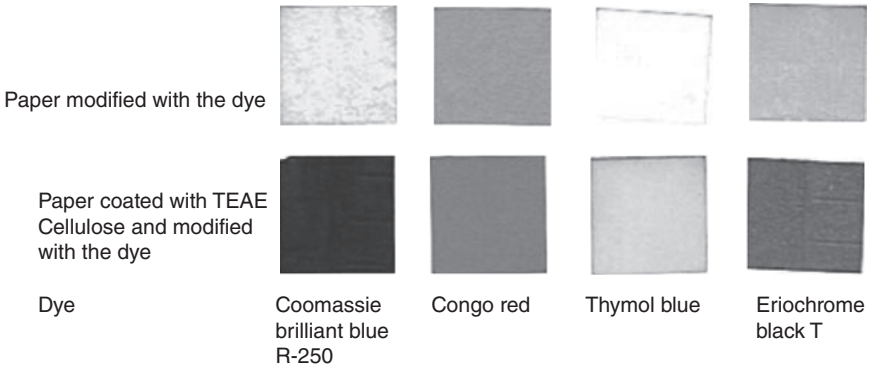

Figure 1.17 Images of printing paper coated with amino cellulose and subsequent coloring. *(See insert for color representation of this figure.)*

spraying and subsequent drying. It turned out that the coated paper possessed a significant antibacterial effect (three different bacteria were tested: *S. aureus*, *K. pneumoniae und Pseudomonas aeruginosa*) [48]. Moreover, the coated paper possessed a much better affinity to color (Figure 1.17).

1.4 Amino Cellulose Carbamates

1.4.1 Synthesis

Recently, an alternative synthetic path for soluble amino group containing cellulose derivatives was established. The reaction of cellulose carbonates, obtained from the biopolymer and chloroformates, with an amino group containing compound yields carbamates and the corresponding alcohol is cleaved off. In particular, cellulose phenyl carbonates have been employed in preparative scale [49]. The efficiency of this reaction (aminolysis) is characterized by the ratio of the DS values after and before the conversion in percent. At least 90% of carbonate moieties are converted into carbamate and for some examples, even a complete conversion was found applying primary amines. However, secondary amines led to cross-linking, while anilines like *p*-toluidine are not reactive due to their low basicity and nucleophilicity. Due to this fact, a synthesis strategy without any protection of an amine group yields a soluble product possessing terminal amino moieties by applying *p*-aminobenzylamine (ABA), which possesses a reactive as well as an inert amino group [50] (Figure 1.18).

The aminolysis of cellulose phenyl carbonates with low DS values (<1.2) usually results in cross-linking. To obtain soluble products, the primary hydroxyl groups (position 6) of cellulose have to be protected prior to carbonate formation, for instance by the trityl group. Nevertheless, the conversion into the carbamate is less efficient for phenyl carbonates derived from secondary hydroxyl groups.

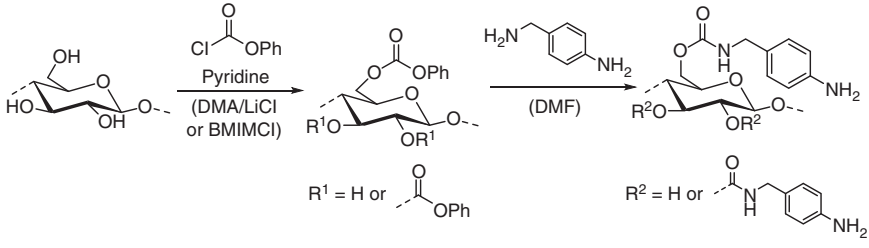

Figure 1.18 Reaction scheme of the synthesis of cellulose phenyl carbonate (first step) and cellulose carbamate by aminolysis applying *p*-aminobenzylamine (second step).

The conversion of cellulose carbonates with aliphatic diamines may result in cross-linking caused by the high reactivity of both amino groups. Thus, the reaction of mono-protected diamines [51, 52] yields soluble cellulose carbamates. The *N*-Boc-ω-aminoalkylcellulose derivatives obtained were subsequently treated under acidic conditions [50].

1.4.2 Properties

ω-Aminocellulose carbamates are able to form layers on a gold surface depending on the pH value. It turned out that no adsorption takes place at pH 5, but a multilayer and aggregates occur on the surface at a pH value of 7 as revealed by QCM-D and AFM. However, at a pH of 10, a rigid and thin film is formed. The adsorbed mass and the RMS roughness of the layer increase with increasing degree of polymerization [53] (Figure 1.19).

In order to immobilize antibodies for an immunoassay, layers of ω-aminocellulose carbamates were applied on polyethylene-sintered filters as support material. Anti-h C-reactive protein (CRP) 6404 antibodies could

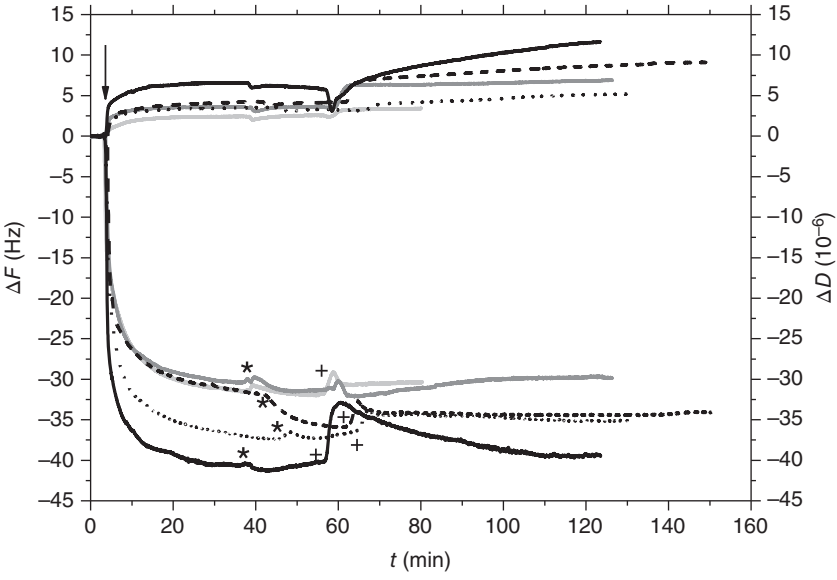

Figure 1.19 Change in frequency and dissipation (third overtone) as function of time during adsorption of ω-aminocellulose carbamates at pH 7; ω-aminoethylcellulose carbamate with low (light gray), medium (gray), and high degree of polymerization (black); ω-amino butylene (dashed), and ω-amino 2,2′-(ethylenedioxy)bis(ethylene) cellulose carbamate (dotted). Addition of sample (arrow) and start of rinsing (*buffer, +water) are marked.

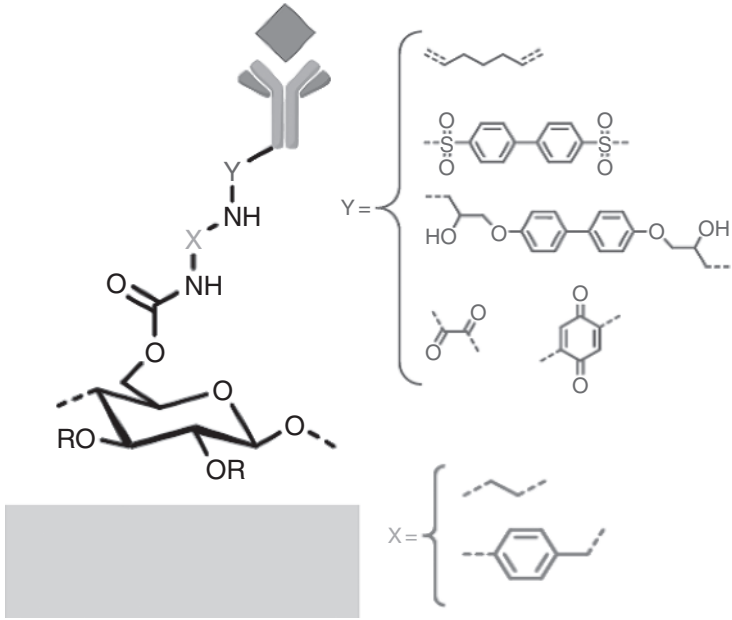

Figure 1.20 Schematic composition of a rapid flow-through immunoassay based on coatings of ω-amino cellulose carbamate.

be attached covalently to the support using different coupling reagents to optimize their activity. Thus, the CRP determination could be carried out by a very sensitive rapid flow-through immunoassay, which shows a detection limit of 5 ng CRP ml^{-1} and a detection range of 5–250 ng CRP ml^{-1}. The activity of the immobilized antibody could be optimized by the kind of aminocellulose carbamate and the coupling structure [54] (Figure 1.20).

ω-Aminoethyl- and ω-aminoethyl-*p*-aminobenzyl cellulose carbamate exhibit a bactericide and fungicide activity *in vitro* [55]. The ω-aminoethylcellulose carbamate possess a strong activity against *Candida albicans* and *S. aureus* (IC_{50} of 0.02 and 0.05 mg ml^{-1}). The antimicrobial activity and biocompatibility could be improved by ABA as additional substituent. The mixed cellulose carbamate exhibits a high biocompatibility (LC_{50} of 3.18 mg ml^{-1}) and forms films on cotton and polyester, which exhibit a strong activity against *S. aureus* and *K. pneumoniae*. The coating of textiles may be applicable in medical devices such as wound dressings. These cellulose carbamates represent alternative substances in healthcare and other applications, where antimicrobial surfaces are needed to prevent the spread of microbial infections.

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