Amino cellulose sulfate: synthesis, characterization and physicochemical behavior

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Abbreviations

ACS	amino cellulose sulfate
AEA	6-deoxy-6-(*ω*-aminoethyl)amino moiety bonded to cellulose backbone
AECS	6-deoxy-6-(*ω*-aminoethyl)amino cellulose-2,3(6)-O-sulfate
AECS-Vis	viscose fibers coated with 6-deoxy-6-(*ω*-aminoethyl)amino cellulose-2,3(6)-O-sulfate
AGU	anhydroglucopyranose unit
APTT	activated partial thromboplastin time
ASTM	american Society for Testing and Materials
BAEA	6-deoxy-6-(2-(bis-N´,N´-(2-aminoethyl) aminoethyl)) amino moiety bonded to cellulose backbone
BAECS	6-deoxy-6-(2-(bis-N´,N´-(2-aminoethyl) aminoethyl)) amino cellulose-2,3(6)-O-sulfate
BAECS-Vis	viscose fibers coated with 6-deoxy-6-(2-(bis-N´,N´-(2-aminoethyl) aminoethyl)) amino cellulose-2,3(6)-O-sulfate
B-Vis	non-coated viscose fibers
°C	degree Celsius
Cadoxen	cadmium ethylene diamine hydroxide $[Cd(H_2N\text{-}(CH_2)_2\text{-}NH_2)_3](OH)_2$
CFU	colony forming units
CS	cellulose sulfate
Cuam	copper ammonium hydroxid $[Cu(NH_3)_4](OH)_2$
Cuen	copper ethylene diamine hydroxid $[Cu(H_2N\text{-}(CH_2)_2\text{-}NH_2)_2](OH)_2$
DAE	1,2-diaminoethane
DMAc	*N,N*-dimethyl acetamide
DMF	dimethylformamid

DMSO	dimethylsulfoxid
DMSO-d_6	deuterated dimethylsulfoxide
DP	degree of polymerization
DP_n	number average degree of polymerization
DS	degree of substitution
DP_w	weight average degree of polymerization
DS_{AEA}	degree of substitution of 6-deoxy-6-(*ω*-aminoethyl)amino moiety bonded to cellulose backbone
DS_{BAEA}	degree of substitution of 6-deoxy-6-(2-(bis-N′,N′-(2-aminoethyl) aminoethyl)) amino moiety bonded to cellulose backbone
DS_{Sulf}	degree of substitution of sulfate groups bonded to cellulose backbone
DS_{Tos}	degree of substitution of tosyl groups bonded to cellulose backbone
δ in FT-IR	in plane bending vibration
δ in NMR	chemical shift in parts per million (ppm)
EA	elemental analysis
Fig	figure
EP	equivalence point
EtOH	ethanol
FT-IR	fourier transform infrared spectroscopy
FWHM	full width at half-maximum intensity
h	hour
HPC	hydroxypropylcellulose
IEP	isoelectric point
MCC	microcrystalline cellulose
MTG	6-O-tosyl-a-Dglucopyranoside

NMR	nuclear magnetic resonance
ν	stretching vibration
$\tilde{\nu}$	number of wavelengths in (cm^{-1})
ν(as)	asymmetric stretching vibration
ν(sy)	symmetric stretching vibration
PDADMAC	polydiallyldimethylammonium chloride
p*K*a	logarithmic constant of acid dissociation
PVSNa	poly(vinylsulfonic acid, sodium salt)
PZC	point of zero charge
RSF	relative sensitivity factor
SEC	size exclusion chromatography
S_N	nucleophilic substitution
SPC	sulfur trioxide pyridine complex
TAEA	tris(2-aminoethyl) amine
T-Blue	toluidine Blue
TBAF	tetrabutylammonium fluoride
TC	tosyl cellulose
TCS	tosyl cellulose sulfates
TEA	triethylamine
TEOA	triethanolamine
TEOA-HCl	triethanolamine hydrochloride
THF	tetrahydrofuran
TN	total nitrogen analyzer
TosCl	*p*-toluenesulfonyl chloride
Tosyl	*p*-toluenesulfonyl

UV	ultraviolet
UV-Vis	ultraviolet–visible spectroscopy
WS	water solubility
XPS	x-ray photoelectron spectroscopy
ZP	zeta potential

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1. Introduction

Increasing attention towards sustainable and renewable resources has evoked a great deal of research activity in recent years (Belgacem and Gandini 2008). In this context, the focus of attention is natural polymers as a source of sustainable and renewable components for the generation of novel materials. Cellulose is by far the most abundant natural resource (Klemm et al. 2005). It is biodegradable, renewable, non-toxic and cheap. Cellulose can be modified by a variety of chemical reactions. There is therefore, ever increasing interest in the use of cellulose as a starting material for novel highly engineered products (Klemm et al. 1998a; Heinze et al. 2006). With respect to studies on the chemical modification of cellulose (Schubert et al. 2011; Koschella et al. 2006; Liebert and Heinze 2004), the interest has primarily focused on nucleophilic displacement (S_N) reactions (Petzold-Welcke et al. 2009). It was found that the *p*-toluenesulfonic acid ester of cellulose (tosyl cellulose) is a very promising starting material for S_N reactions. Moreover, it is feasible to modify the remaining hydroxyl groups of tosyl cellulose with various ester moieties, including negatively charged sulfate moieties without influencing the presence of the tosylate group (Heinze and Rahn 1996). Thus, polyampholytes carrying both types of ionic groups within one macromolecule can be synthesized by replacing the tosylate groups via S_N reactions. The polyampholytes produced may display unique physicochemical characteristics and have great potential for various applications, including protein separation and purification, binding and recovery of metal ions, enhanced oil recovery, and adsorption on viscose fibers similar to chitosan (Kudaibergenov and Ciferri 2007; Strnad et al. 2010).

The aim of the present work is to provide a synthesis pathway to novel cellulose-based polyampholytes. The functionalization of cellulose with *p*-toluenesulfonyl (tosyl) moieties and the subsequent introduction of sulfate moieties by the conversion of the remaining hydroxyl groups with sulfur trioxide pyridine complex yields a reactive amphiphilic polyelectrolyte, namely tosyl cellulose sulfate (Heinze and Rahn 1996). Tosyl cellulose and tosyl cellulose with

additional hydrophobic functional groups (e.g. acetate, benzoate, carbanilate) are accessible to nucleophilic displacement reactions with multifunctional amines (Tiller et al. 1999; Tiller et al. 2001; Berlin et al. 2000; Berlin et al. 2003; Jung and Berlin 2005). However, the nucleophilic displacement of tosyl groups in sulfated cellulose polyelectrolytes has not yet been reported. In this thesis, the synthesis of tosyl cellulose sulfate, and the replacement of the tosyl groups by different multifunctional amines was carried out and the structures of the novel ampholytic products (namely 6-deoxy-6-amino cellulose sulfate derivatives) were characterized by means of elemental analysis, ^{13}C-NMR-, FT-IR-, and UV-Vis spectroscopy. Moreover, the solubility of the samples in water at different pH values, the molecular weights and physicochemical behavior of the samples in aqueous solution were studied. 6-Deoxy-6-amino cellulose sulfate products were adsorbed on cellulose fibers, thereby conferring amphoteric characteristics. The functional groups displayed on the surface of the coated fibers and the electro-kinetic behavior of the coated fibers were characterized by means of different techniques and methods. Finally, studies on the antimicrobial and antithrombogenic efficiencies of the samples in aqueous solution and adsorbed on cellulose fibers were carried out.

2. Literature review

2.1. Cellulose

Cellulose is a valuable natural product. Human civilization uses it for a variety of applications in fields as diverse as medicine, pharmaceutics, cosmetics, clothing, construction, heating and food. There are only a few facets of human life which are not directly connected to cellulose or one of its derivatives. Thus, it is not surprising that a large amount of cellulose related research has been accomplished; indeed, two Nobel Prizes have been awarded for cellulose related research (Harris et al. 2010). Cellulose was characterized for the first time in various plants' tissues by the French plant scientist Anselme Payen (Payen 1838). He found that after treating the plant tissues with acids and ammonia, a fibrous solid could be extracted in water, alcohol, and ether. He determined the molecular formula ($C_6H_{10}O_5$) of the extracted fibrous using elemental analysis, and observed its structural isomerism with starch. Subsequently, it was named as (Cellulose) by the French Academy (Brongniart et al. 1839). Cellulose is an essential ingredient of all woody plants (**Fig. 1**), and is the most abundant, biodegradable and renewable polymer in the world. The annual amount of naturally produced cellulose is around 1.5 x 10^{12} tons (Klemm et al. 2005).

2.1.1. Cellulose occurrence and extraction

The most important source of cellulose is plants (Klemm et al. 2002). Sources of cellulose such as lignocellulosic material in wood, agricultural residues, water plants and grasses also contain other components such as hemicelluloses, lignin and small amounts of extractives (Hon 1996). However, the cellulose obtained for commercial uses tends to be harvested from sources such as cotton and wood due to their relative purity (**Table 1**).

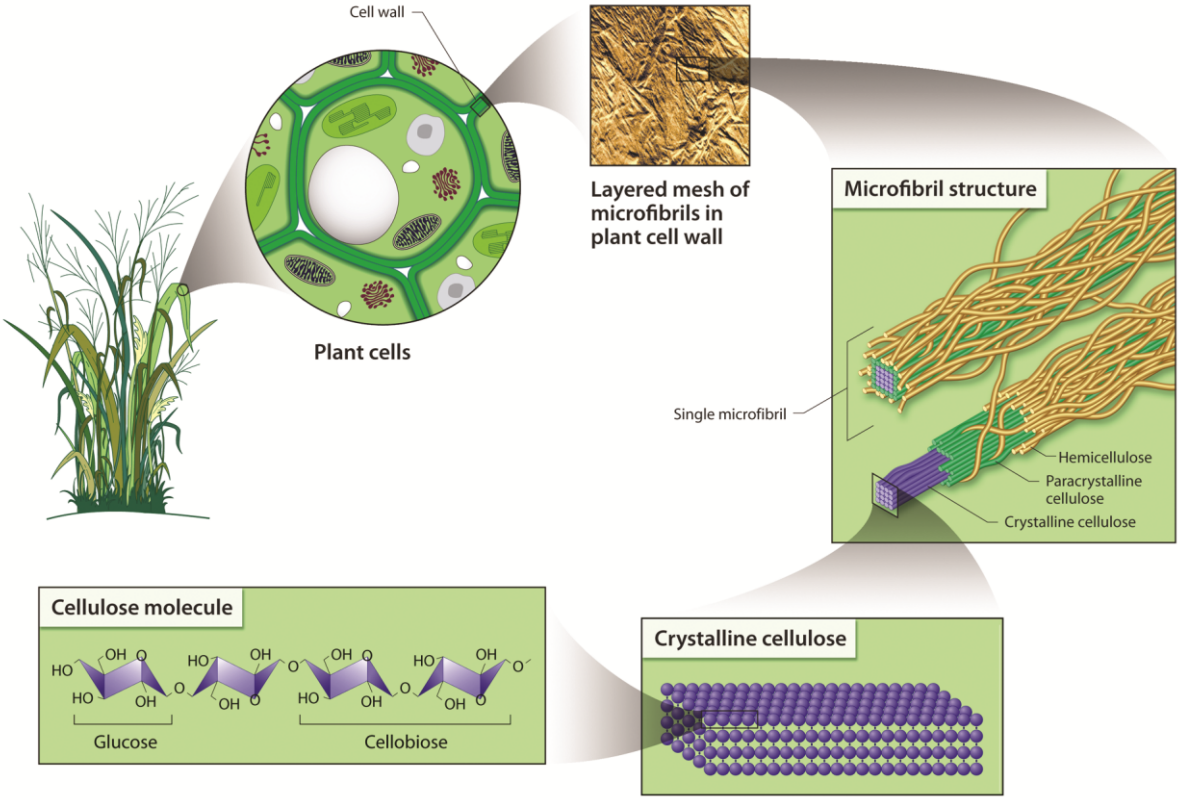

Fig. 1. Cellulose microfibril structure according to the office of biological and environmental research of the U.S. Department of Energy Office of Science. science.energy.gov/ber/

It is also noteworthy that pure cellulose can be produced on a laboratory and semi-industrial scale, by acetic acid producing bacteria, such as *Gluconacetobacter, xylinum* and *Acanthamoeba castellani* (Tarchevskiĭ and Marchenko 1991). Algae such as *Valonia ventricosa* and *Chaetamorpha melagonicum* are also sources of highly crystalline cellulose, which have been used for studying polymorphism in polymers (Sugiyama et al. 1990; Yamamoto et al. 1989; VanderHart and Atalla 1984; Isogai et al. 1989). There is cellulose of animal origin and also cellulose, which found in fungal cell (Van Daele et al. 1992; Johansson et al. 1989).

Table 1. Chemical composition of some typical cellulose-containing material according to Hon 1996.

Source	Composition (%)			
	Cellulose	Hemicellulose	Lignin	Extract
Hardwood	43 – 47	25 – 35	16 – 24	2 – 8
Softwood	40 – 44	25 – 29	25 – 31	1 – 8
Bagasse	40	30	20	10
Coir	32 – 43	10 – 20	43 – 49	4
Corn cobs	45	35	15	5
Corn stalks	35	25	35	5
Cotton	95	2	1	0.4
Flax (retted)	71	21	2	6
Flax (unretted)	63	12	3	13
Hemp	70	22	6	2
Henequen	78	4 – 8	13	4
Istle	73	4 – 8	17	2
Jute	71	14	13	2
Kenaf	36	21	18	2
Ramie	76	17	1	6
Sisal	73	14	11	2
Sunn	80	10	6	3
Wheat straw	30	50	15	5

Since cellulose is isolated from plants, it may contain some byproducts which are not desirable for chemical modification reactions. The industrial extraction and purification of cellulose has led to differing levels of purity from ultra-pure cellulose to cellulose which contains byproducts (Klemm et al. 2002). **Table 2** gives some examples of such cellulose materials.

Table 2. Carbohydrate composition and degree of polymerization (DP) of some cellulose samples. (adapted from Heinze 1998).

Sample	Producer	Carbohydrate composition (%)			DP
		Glucose	Mannose	Xylose	
Avicel	Fluka	100	-	-	280
Sulfate pulp V-60	Buckeye [a)]	95.3	1.6	3.1	800
Sulfate pulp A-6	Buckeye	96.0	1.8	2.2	2000
Sulfite pulp 5-V-5	Borregaard [b)]	95.5	2.0	2.5	800
Linters	Buckeye	100	-	-	1470
Linters	Buckeye	100	-	-	2000

[a)] Buckeye Cellulose Corp., 1001 Tillman Street, Memphis/Tennessee 38108 – 0407, USA;

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

In **Table 2**, it is shown that the degree of polymerization (DP) and the molecular weight of cellulose (molecular weight = DP×162 g mol^{-1}) vary widely according to the sources and the treatment methods.

Extraction of cellulose involves treatment of the cellulosic materials with alkalis or bisulfites to separate the lignin and to extract the hemicellulose. Different extraction procedures can be performed, with each method possessing different advantages and disadvantages related to the amount, quality, composition and final properties of the resulting cellulose (Morán et al. 2008). The process can be summarized as digestion, removing most of the lignin as lignosulfonic acid (sulfite process) or as alkali or thiolignin (graft and sulfate process respectively), and subsequent bleaching steps for eliminating most of the residual lignin. The average chain length and the purity of the wood pulp obtained can be adjusted according to the end-use demands by controlling the digestion and bleaching steps (Klemm et al. 1998a).

2.1.2. Cellulose structure and reactivity

Cellulose is rigid, highly crystalline, and insoluble in common organic solvents, which make it an ideal material for structural engineering (Hon 1995; Varshney and Naithani 2011). Cellulose is a polydisperse and linear polymer. Its basic monomeric unit is D-anhydroglucopyranose unit (AGU). AGUs are linked together by β-(l→4)-glycosidic bonds and every two glucose units are assembled in a group forming an isotactic polymer composed of cellobiose dimers. **Fig. 2** shows the typical molecular structure of the cellulose chain containing cellobiose dimer units. In every pyranose ring, the $–CH_2OH$ and –OH groups and the glycosidic bonds are all equatorial with respect to the mean planes of the rings, which means that pyranose rings possess a $^4C_1$ conformation. The C-1 end has reducing properties, while the glucose end group with a free C-4 hydroxyl group is non-reducing. The cellulose molecule takes a form like a flat ribbon when it is fully extended and the hydroxyl groups which protrude laterally form inter- and intramolecular hydrogen bonds. The linear integrity of the cellulose chains and the reactivity of the hydroxyl groups are both affected by the intramolecular hydrogen bonding between the adjacent AGU rings. Particularly, C-3 hydroxyl group forms a strong hydrogen bond with the ring oxygens on adjacent AGU units. These features are responsible for the supramolecular structure of cellulose and determine its chemical and physical properties (Varshney and Naithani 2011). The solid state of the cellulose has highly ordered crystalline zones beside less ordered amorphous areas. The hydroxyl groups located in the amorphous areas are more available for the reactions than the ones in the more highly ordered crystalline zones (Coffey et al. 1995). The degree of crystallinity of cellulose is defined as the ratio of the amorphous to the crystalline cellulose, and it depends on the pretreatment of the samples and its purity (Fink et al. 1995). For example, celluloses derived from cotton, bacteria and Valonia algae have the degree of crystallinity about 40–45%, 75% and 93%, respectively (Kulshreshtha and Dweltz 1973; Yamamoto and Horii 1993; O'Sullivan 1997). The most important factor which determines the chemical reactivity of cellulose originates from

the existence of three reactive hydroxyl groups within each AGU unit. The primary ones located on position six, and the secondary hydroxyl groups, located on positions two and three (**Fig. 2**). The three hydroxyl groups in each AGU unit, about 31.48% of the cellulose weight, offer considerable possibilities for functionalization. The reactivity of the hydroxyl groups of cellulose depends on the medium in which reaction is carried out. This can be clearly noticed in etherification reactions performed in an alkaline medium where the order of the reactivity OH groups is OH-2 > OH-6 > OH-3 while in the case of esterification reactions, the primary hydroxyl group (OH-6) is preferred (Hon 1996). However, the uniformity of the resulting products is not guaranteed due to the challenge of the regioselectivity of the specific syntheses. The type, distribution, and achieved uniformity of substituents groups clearly determine the properties of the derivatives (Klemm et al. 2002 ; Nicholson and Meritt 1985). The degree of substitution (DS) represents the average number of the hydroxyl groups functionalized by the new substituents. The highest DS that can be reached is three per anhydroglucose unit. Hydroxyl groups of cellulose can be involved in different types of reactions such as esterification, etherification, intermolecular crosslinking reactions, and free radical reactions, particularly in the formation of cellulose graft copolymers. Thus, a large variation of different chemical functionalizations of cellulose can be achieved (Varshney and Naithani 2011). Under appropriate conditions, the regions C-1 and C-4 are involved in degradation processes. The degradation can occur chemically (acid and enzymatic heterolysis, alkaline and oxidative degradation), mechanically (milling, ultrasonic agitation), thermally, and by radiation (UV, high energy radiation) (Klemm et al. 1998a).

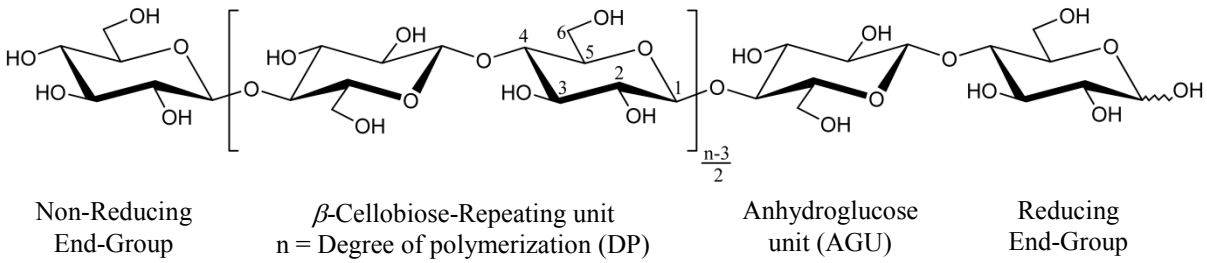

Fig. 2. Molecular structure of cellulose.

2.1.3. Dissolution and regeneration of cellulose

Regenerated cellulose is produced via dissolution. The hydrogen bonds, which form the organized system surrounding the single polyglucan chain, are destroyed during the dissolution process (Heinze and Liebert 2001). The solvents used are classified to non-derivatizing and derivatizing solvents (see **Fig. 3**). Physical dissolution is performed by non-derivatizing solvents such as *N,N*-dimethyl acetamide (DMAc)/LiCl, while derivatizing solvents such as $CF_3COOH/(CF_3CO)_2O$ cause partial functionalization of the cellulose. Both solvents types can be used as media for derivatization and to prepare regenerates of cellulose (Klemm et al. 2002). In 1857, Schweizer reported the first solvent of cellulose, which is cuprammonium hydroxide solution (Schweizer 1857). After that, several solvents were reported and many important reviews have been published containing detailed discussion about known cellulose solvents and their respective mechanism of dissolution (Hudson and Cuculo 1980; Johanson 1985; Augustine and Hudson 1990; Philipp 1993; Dawsey 1994; Heinze and Liebert 1998; Heinze and Glasser 1998). As a result of dissolution of cellulose, various types of regenerated cellulose were derived such as microcrystalline cellulose and viscose fiber.

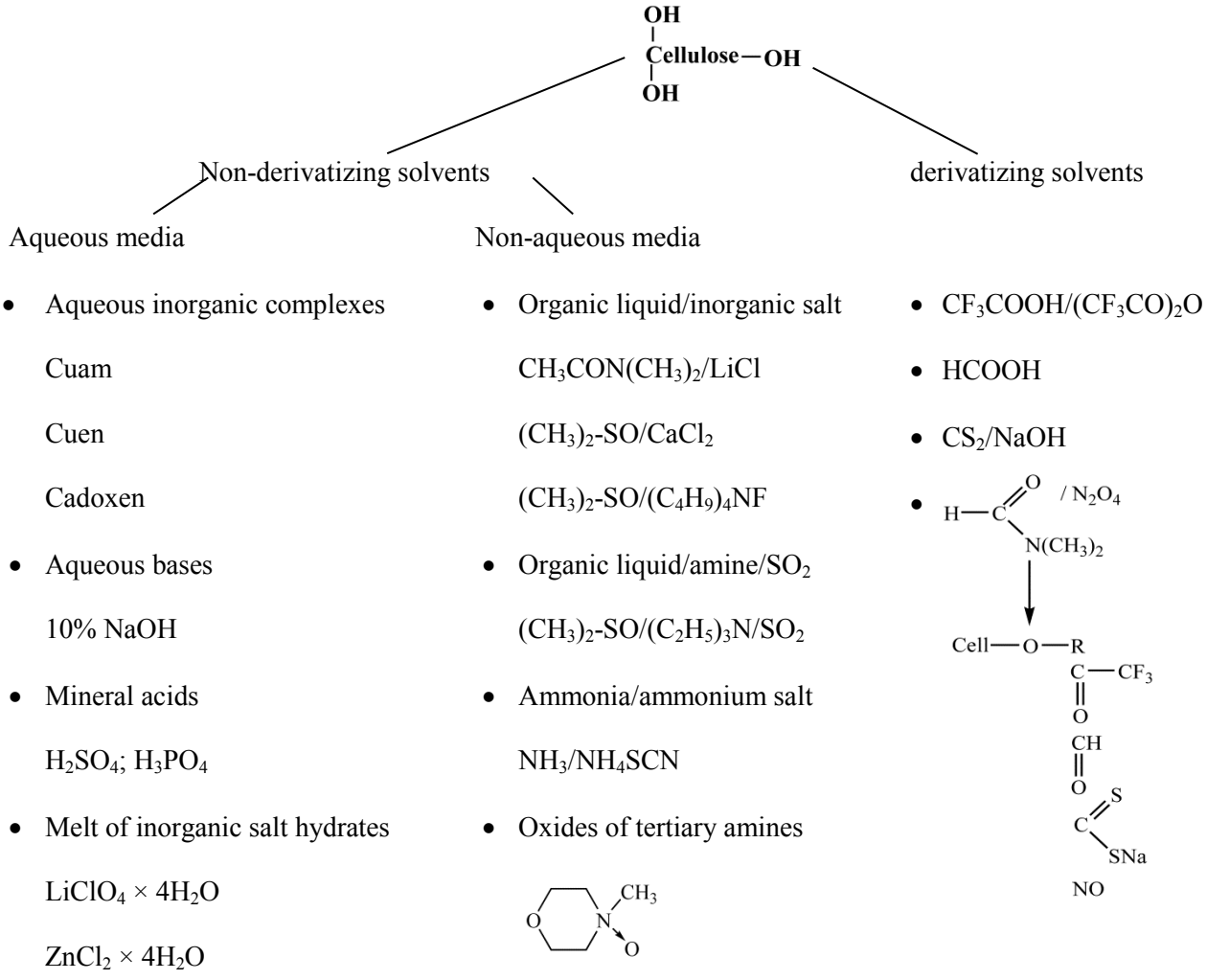

Fig. 3. Classification of typical cellulose solvents. (adapted from Heinze and Liebert 2001)

2.1.3.1. Microcrystalline cellulose

Microcrystalline cellulose (MCC) is produced by acid catalyzed depolymerization of native or the regenerated cellulose. For this purpose, HCl, SO_2 and H_2SO_4 at high temperatures (110 °C) for ca. 15 minutes are used (Battista 1985). The amorphous regions are targeted by the acidic reagents causing degradation, which reduces the degree of polymerization. Only the acid-resistant crystalline regions are saved. After spray drying of the MCC powder, the average particle size range is between 20-90 µm. Another type of MCC (called colloidal MCC) is water

dispersible and possesses a major proportion of colloidal sized aggregates (less than 0.2µm in diameter). For preparation of the colloidal MCC, mechanical energy is needed after the acidic hydrolysis in order to make microfibrils of the required size (French and Bertoniere 1993; Whistler and Daniel 1990).

2.1.3.2. Viscose fiber

In 1891, Charles Cross, Edward Bevan, and Clayton Beadle discovered that cotton or wood cellulose after treatment with alkali and carbon disulfide could be dissolved as cellulose xanthate. The treacle-like yellow solution was called 'viscous cellulose solution' which was later contracted to simply 'viscose'. It was noticed that the viscous cellulose solution could be coagulated in an ammonium sulfate bath and then converted back to pure white cellulose using dilute sulfuric acid. This was known as the viscose process and the first patent on the viscose process was granted to Cross and Bevan in England in 1893 (Cross et al. 1893) (see **Fig. 4**). By 1908, the fiber spun from viscose solution was considered as a key component of the textile industry and still enjoys the unique position of being the most versatile of all artificial fibers (Woodings and Editor 2001). At present, annually, 3 million tons of viscose fibers are produced worldwide through the viscose process (Klemm et al. 2002). There are many different technical procedures for the steps of xanthogenation, dissolution, and thread formation. Details about the chemistry of xanthogenation, transxanthogenation, and dexanthogenation and their influence on the substituent distribution and the solution state were discussed in (Götze 1967; Albrecht 1986; Engelhardt 1995 ; Klemm et al. 1998a).

2.1.3.2.1. Properties of viscose fibers

Viscose fibers are hydrophilic, absorbent, and skin-friendly. They provide good moisture management combined with restricted growth of microorganisms. These fibers are non-melting,

mechanically and chemically stable, but if needed, they may be physically and chemically modified (Roggenstein 2011). Cellulose fibers are the most appropriate to be used as antibacterial next-to-skin fabrics due to their low immunogenicity towards the human body, and the comfort resulting from their high moisture absorption (Di et al. 2011). These features make the viscose fibers one of the most important textile materials worldwide.

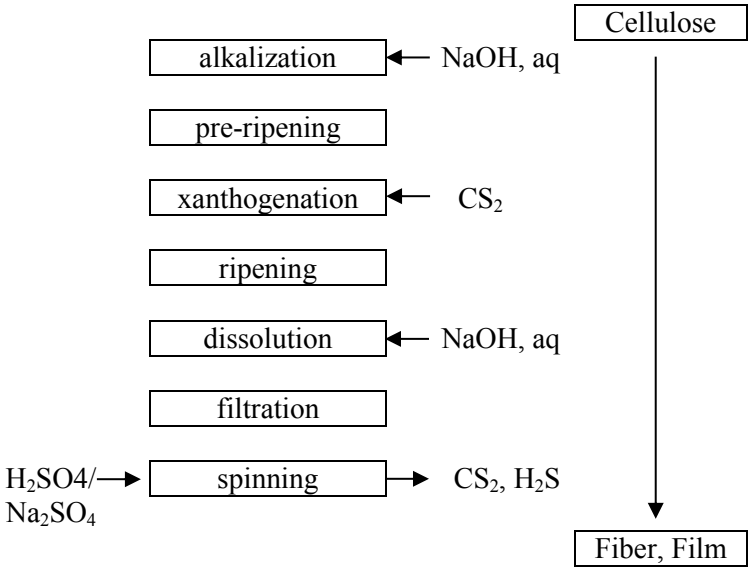

Fig. 4. Viscose process (schematically). (adapted from Klemm et al. 2002)

2.1.3.2.2. Adsorption and characterization of agents on viscose fibers

Surface treatments of cellulose fibers with intelligent functionalities is one of the important research areas nowadays. The aim of that is to improve their existing properties or to add new properties to the fiber's surface, such as improving hydrophobicity for the reduction of water uptake and release, increasing their reactivity towards polymeric matrices in order to prepare high-performance composite materials (Tonoli et al. 2009; Veronovski and Hribernik 2010), grafting of specific sensors at their surfaces to render them as photoluminescent (Kim et al. 2006; Sarrazin et al. 2007), providing their surfaces with amphoteric characteristics, which may further

expand their potential usages by improving their surface adsorbtivity as well as their fiber dyeing capabilities (Ramesh Kumar and Teli 2007), and introducing a bioactive functionality, which may allow the medical use of the cellulose fibers (Yuan Gao and Cranston 2008; Simoncic and Tomsic 2010).

It must be taken into account that before adsorbing any active agent, viscose fibers must be pre-treated with alkali. Thus, the impurities which may lower their hydrophilicity and adsorptivity such as oils, waxes, antistatic agents, lubricants and stiffening additives, originating from the manufacturing process may be removed (Bredereck et al. 1996; Zemljič et al. 2008; Peršin et al. 2004; Peršin et al. 2011). It is known that purified (alkali pretreated) regenerated cellulose fibers are negatively charged due to the presence of carboxyl groups within their structures (Stana-Kleinschek and Ribitsch 1998; Stana-Kleinschek et al. 2001; Stana-Kleinschek et al. 2002). Therefore, an interaction between the negative charges on the fibers and positively charged molecules (e.g. polyelectrolytes) may facilitate their adsorption on the fibers. The process and theoretical aspects of polyelectrolyte adsorption on cellulose fibers have attracted considerable interest over the last 40-50 years, and many reviews have been already published on this topic (Wägberg and Ödberg 1989; Lindström 1989; Ödberg et al. 1993; Swerin and Ödberg 1997). An important example of fiber functionalization is the viscose fibers functionalized by chitosan which renders the surface of the fibers antimicrobial activity. The fibers are typically soaked for 10 minutes in 100 ml of 1% chitosan solution, which is adjusted to pH 3.6 by the addition of 1 M HCl. The adsorption and desorption of the chitosan is analyzed using potentiometric titrations, the spectrophotometric method with dye C.I. acid orange VII, the Kjeldahl technique and polyelectrolyte titrations (Fras Zemljic et al. 2012). In addition to chitosan, several active agents can be introduced to the cellulose fibers to be used as antimicrobial agents (such as silver, quaternary ammonium salts, polyhexamethylene biguanide, triclosan, dyes and regenerable *N*-halamine compounds and peroxyacids) (Yuan Gao and Cranston 2008).

Evaluation of the efficiency of absorption on the surface of the viscose fibers is achieved via analysis of the functional groups on the fibers' surface before and after the adsorption has taken place. X-ray photoelectron spectroscopy is used for the determination of the surface element content of carbon, nitrogen, oxygen and sulfur (Buchert et al. 2001; Fras et al. 2005). For studying the physicochemical and the electro-kinetic behavior of the fibers, techniques such as Conductometric-, potentiometric and polyelectrolyte titrations are used (Fras et al. 2004; Cakara et al. 2009; Wägberg and Ödberg 1989; Laine et al. 1996), as well as conventional spectroscopic techniques such as the methylene-blue method, and zeta-potential measurements of the fibers as a function of pH (Klemm et al. 1998a; Stana-Kleinschek et al. 2001; Reischl et al. 2006).

2.2. Chemical modification of cellulose

Functionalization of cellulose can be performed either heterogeneously or homogenously. A successful uniform heterogeneous functionalization with high DS value needs efficient cellulose pretreatment in order to decrease the interfibrillar bonding caused by hydrogen bonds (Klemm et al. 2002). The most appropriate procedure used for activation of cellulose to be prepared for heterogeneous functionalization is swelling in polar liquids such as dimethylsulfoxid (DMSO), ethanol (EtOH), NH_3, and H_2O, R_4NOH/H_2O, or on the lab-scale, NH_3 and with transitory formation of additional compound with $NaOH/H_2O$ (mercerization for technical etherification in particular). Several typical examples about the very fast formation of alkali cellulose with aqueous NaOH were explained in (Klemm et al. 1998a). Dissolution of cellulose (see chapter 2.1.3) opened the way for functionalization of cellulose under homogenous conditions. By using protecting group techniques, a regioselective functionalization of cellulose for producing of novel types of cellulose derivatives could be widely performed (Qi et al. 2012; Susann et al. 2010; Nagel et al. 2010; Schlufter et al. 2006; Heinze and Rahn 1996; Rahn et al. 1996).

2.2.1. Esterification of cellulose

Various pathways for the esterification of cellulose have been investigated. They are one of the most versatile classes of transformations of the biopolymer. On a technical scale, cellulose derivatives are synthesized by heterogeneous procedures (Heinze et al. 2006). Esterification of cellulose involves the reaction of the primary and secondary hydroxyl groups of cellulose. The technicality which makes esterification reactions of cellulose different from those occurring on low molecular weight alcohols arises from the macromolecular structural aspects of cellulose (Wilks 2001). Hydrogen bonds reduce the solubility of cellulose by linking the individual chains and forming complex structures. Derivatization of cellulose into esters modifies the solubility of resultant cellulosic derivate and changes many of the properties of the cellulose. Esterificationcan of cellulose can be carried out using mineral acids, organic acids or their anhydrides with the aid of dehydrating substances. The resulting esters are either organic or inorganic (Shelton 2004; Balser et al. 2000) (see **Fig. 5**). Because of the equilibrium adjustment, it is not guaranteed that the esterification reactions take place according to simple stoichiometric rules. The maximum degree of substitution of 3 is difficult to be achieved. Only under carefully controlled conditions can a triester be obtained. The most preferred hydroxyl group for the esterification reaction is the primary one which is located on C-6, while the secondary hydroxyl groups on the C-2 and C-3 positions of the AGU react slower due to their steric hindrance (Balser et al. 2000). In spite of the variety of cellulose esters which can be produced, the focus in next two chapters will be only on the cellulose esters which are used in this work.

2.2.1.1. Esters of cellulose with organic acids

The synthesis of cellulose esters of organic acids is obtained by the esterification of hydroxyl groups which are commonplace in organic chemistry. Except in some cases, the reactivity of the

organic acid itself even when present at a large excess is insufficient for performing an esterification reaction of cellulose.

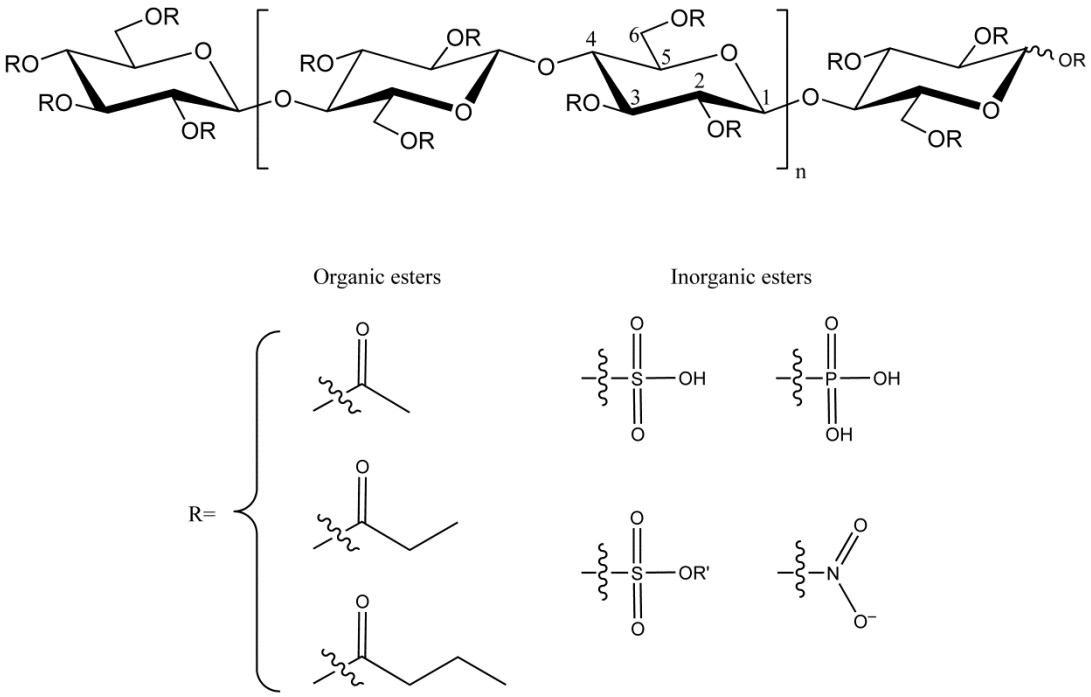

Fig. 5. General structure of cellulose esters. (adapted from Shelton 2004)

Therefore, carboxylic acid derivatives such as acid anhydrides or acid chlorides are used as reagents. Generally, the esterification of the hydroxyl groups is achieved under acidic conditions which may lead to hydrolytic cleavage of the backbone of cellulose. Only under highly alkaline conditions can irreversible saponification of the ester groups take place. A broad variety of cellulose esters has been synthesized such as cellulose formate, cellulose acetate, cellulose esters of higher aliphatic acids, esters of cellulose with substituted mono-, di- and tricarboxylic aliphatic acids, cellulose esters with aromatic acids, esters of cellulose carrying sulfonic or phosphonic acid groups, phenyl carbamates of cellulose (Klemm et al. 1998b). The focus in this work will be on the *p*-toluenesulfonyl ester of cellulose (Tosyl cellulose) which is used in the experimental part.

2.2.1.1.1. Tosyl cellulose

Esters of cellulose with *p*-toluenesulfonic acid, tosyl celluloses form versatile intermediates in the organic chemistry of cellulose derivatization. In other words, the tosyl groups are considered as a protective group as well as a very good leaving group for subsequent nucleophilic substitution reactions, which allow for the introduction of different moieties to the cellulose chain (Petzold-Welcke et al. 2009; Klemm et al. 1998b; Heinze et al. 2001). Tosyl cellulose can be synthesized heterogeneously as well as homogenously and the higher reaction rate, in both cases, has been clearly noticed at C-6 position as compared with those at C-2 and C-3 (Takahashi et al. 1986; Petzold-Welcke et al. 2009; Rahn et al. 1996).

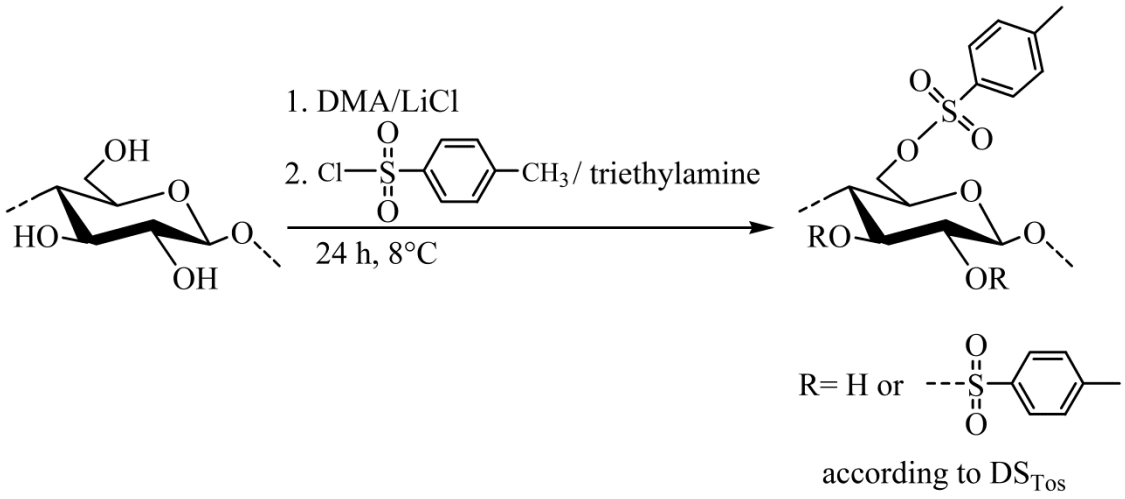

Fig. 6. Synthesis path for the molecular structure of tosyl cellulose. (adapted from Rahn et al. 1996)

Heterogeneously, tosyl cellulose has been prepared by reacting cellulose suspensions with large excesses of the reagent (up to 40: 1 mol of *p*-toluenesulfonyl chloride per mol of AGU) in pyridine for a long reaction time (ca. days) between room temperature and 80 °C (Hess and Ljubitsch 1933; Honeyman 1947). The heterogeneous procedure has several disadvantages; among them are the long reaction times, the high amounts of the reagents needed for the reactions, chlorination, formation of aminodesoxy groups and the poor solubility of the products.

The disadvantages of the heterogeneous process have been avoided through the synthesis of tosyl cellulose in a homogenous system. The procedure has been described in (Rahn et al. 1996) (see **Fig. 6**). A non-derivatizing solvent system, e.g. DMAc/LiCl, has been used for dissolving cellulose with DP values ranging from 280 to 1020. Ratios between 0.6-9.0 mol of tosyl chloride/mol of AGU were used to synthesize tosyl celluloses of DS values between 0.4 and 2.3 in the presence of triethylamine (TEA) as the base (with negligible incorporation of chlorodeoxy groups) for a period of 24 h at 8 °C (see **Table 3**).

Table 3. Reaction of cellulose with tosyl chloride (TosCl) in DMAc/LiCl for 24 h at 8 °C. (adapted from Rahn et al. 1996).

Cellulose	Reaction conditions				Reaction product		
		Molar ratio					
	DP	AGU	TosCl	TEA	DS_{Tos}	S (%)	Cl (%)
Microcrystalline	280	1.0	1.8	3.6	1.36	11.69	0.47
		1.0	4.5	9.0	2.30	14.20	0.43
Spruce sulfite pulp	650	1.0	1.8	3.6	1.34	11.68	0.44
		1.0	9.0	18.0	1.84	13.25	0.49
Cotton linters	850	1.0	0.6	1.2	0.38	5.51	0.35
		1.0	1.2	2.4	0.89	9.50	0.50
		1.0	2.1	4.2	1.74	12.90	0.40
		1.0	3.0	6.0	2.04	13.74	0.50
Beech sulfite pulp	1020	1.0	1.8	3.6	1.52	12.25	0.43

As shown in **Fig. 7**, the mechanism for the reaction of cellulose with sulfonic acid chloride in DMAc/LiCl depends on the organic base used in the reaction. In more detail, sulfonic acid chloride reacts with DMAc in a Vilsmeier-Haack-type reaction and form O-(*p*-toluenesulfonyl)-

N,N-dimethylacetiminium salt **I**. In the presence of organic bases such as TEA (p*K*a 10.65) or DMAc (p*K*a 9.70), intermediate **I** reacts with the spieces **V** which leads to the formation of cellulose sulfonic acid esters **VI** without side reactions. In contrast, weaker bases such as Py (p*K*a 5.25) or *N,N*-dimethylaniline (p*K*a 5.15) react with **I**, yielding a more reactive *N,N*-dimethylacetiminium salt **II** compared with **V**. Thus, at high temperatures, **II** can form chlorodeoxy compounds **III** or after aqueous workup, it yields acetylated cellulose **IV,** and both products are undesired side products (McCormick et al. 1990; Heinze et al. 2006).

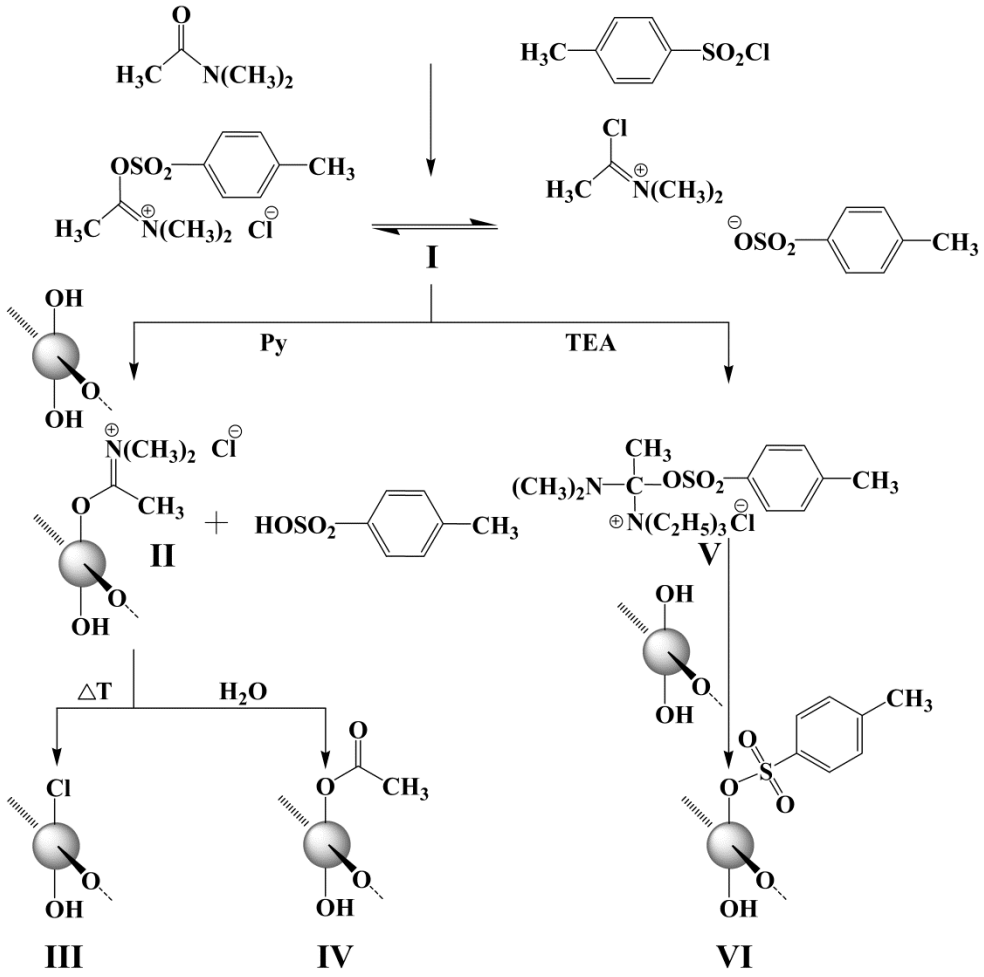

Fig. 7. Mechanism for the reaction of cellulose with tosyl chloride in DMAc/LiCl depending upon the presence of different organic bases. (adapted from McCormick et al. 1990; Heinze et al. 2006).

The synthesized products are soluble in DMSO in all DS_{Tos} values obtained herein, whereas in the case of other solvents such as dimethylformamid (DMF), acetone, tetrahydrofuran (THF) or chloroform the solubility depends on the DS_{Tos}. Tosylate can be employed as a protecting group for synthesis mixed esters of cellulose by esterification of the free hydroxyl groups. Besides this, amphiphilic esters such as phthalates, trimellitates and sulfates of cellulose tosylates with unconventional solubility properties can be synthesized. For example, the introduction of sulfate groups to tosyl cellulose results in water-soluble cellulose sulfate esters with tosylate groups capable of being substituted through subsequent nucleophilic reactions facilitating the design of new supramolecular cellulosic structures (Heinze and Rahn 1996; Heinze et al. 2001; Petzold-Welcke et al. 2009; Klemm et al. 1998b; Heinze et al. 2006).

2.2.1.2. Esters of cellulose with inorganic acids

In spite of the large number of inorganic acids available only few of them have been used in the esterification of cellulose. In this context, the most common inorganic acids used for cellulose esterification are oxygen-containing acids of the elements nitrogen, phosphorus, sulfur and boron. Thus, several esters of cellulose were produced such as cellulose nitrate, cellulose nitrite, cellulose sulfates, cellulose phosphate, cellulose dithiocarbonic acid ester, and cellulose borates (Klemm et al. 1998b; Heinze et al. 2006) (see **Fig. 8**). Since cellulose sulfate is involved in the recent work, details about it will be discussed below.

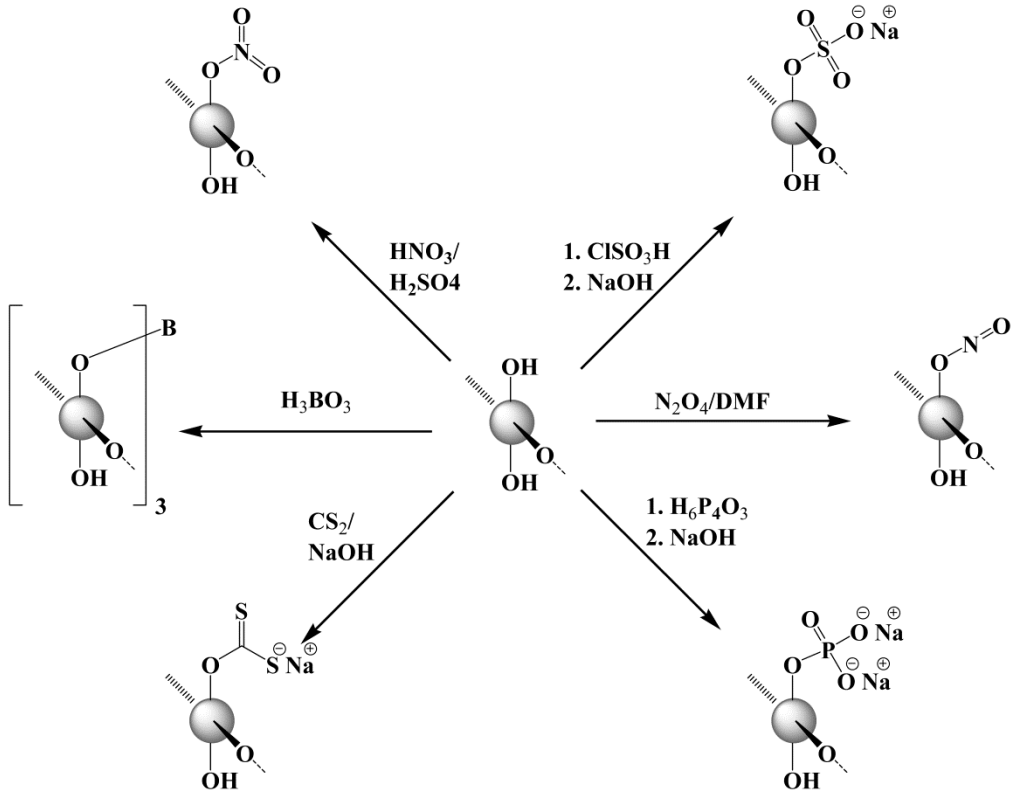

Fig. 8. Examples of polysaccharide esters of inorganic acids. (adapted from Heinze et al. 2006)

2.2.1.2.1. Cellulose sulfate

The term (cellulose sulfate) refers to the sulfuric acid half-ester of cellulose. It can be obtained by esterification of hydroxyl groups of cellulose according to:

$$\text{Cell-OH} + SO_3 \longrightarrow \text{Cell-OSO}_3\text{H}$$

$$\text{Cell-OH} + XSO_3H \longrightarrow \text{Cell-OSO}_3\text{H} + XH$$

(X = H_2N, HO, Cl)

The resultant acid half-esters can be converted to neutral sodium salts that are soluble in water when the DS is above of 0.2-0.3 depending on the distribution of the ester moieties. Cellulose sulfate can be synthesized by displacing of very labile nitrite group from its position in the AGU by using various sulfating agents. In this way, a reaction system of cellulose dissolved with an excess of N_2O_4 in DMF (> 3 mol of N_2O_4/mol of AGU) to a cellulose trinitrite and containing an

excess of N_2O_4 and HNO_3 as further components, can be directly sulfated without isolation of the cellulose trinitrite (Schweiger 1974; Wagenknecht et al. 1993). Cellulose has also been sulfated by using a complex of sulfur trioxide reacted with DMSO achieving a DS of sulfation up to 2 (Whistler et al. 1967). The most common sulfating agents used are sulfuric acid, sulfur trioxide or chlorosulfonic acid which are used as the only reaction component besides cellulose, or in combination with alcohols, amines or inert media like chlorinated hydrocarbons. By adaption of the reaction conditions such as time, temperature and molar ratio of agent to AGU, a range of DS of sulfate group between 0 and 3 can be achieved. The cellulose chain during sulfation is commonly degraded due to hydrolytic cleavage of the glycosidic bonds (Klemm et al. 1998b). Cellulose sulfate can be synthesized through three routes; heterogeneously using an activated cellulose suspension, homogeneously starting with partially substituted cellulose derivatives and by the displacement reaction of an ester or ether group already displayed on the macromolecule. (Gohdes and Mischnick 1998; Wagenknecht et al. 1993; Klemm et al. 1998b). Cellulose sulfate possesses a variety of interesting properties, such as solubility, rheological behavior, different interactions with low or high molecular weight cations, anticoagulant activity and antiviral activity (Wagenknecht et al. 2005). The focus of this work is on the homogeneous synthesis of cellulose sulfates starting from partially functionalized cellulose esters with a primary substituent acting as a protecting group. Thus, in a suitable dipolar aprotic medium, the cellulose can be sulfated using conventional sulfating agents. The sulfation process targets only the free hydroxyl groups (Klemm et al. 1998a). Regioselective sulfation offers, in this case, a route to tosyl cellulose sulfates with a well-defined site-selective distribution of the sulfate groups within the AGU as explained in Heinze and Rahn 1996. The tosyl group of the partially esterified sulfonic acid ester of cellulose (tosyl cellulose) plays the role of a protecting group in a subsequent sulfation, as it is stable under the reaction conditions for sulfation using sulfur trioxide pyridine complex (SPC) in DMAc. After isolation of the product, it is neutralized with aqueous NaOH forming the sodium sulfate half-ester (see **Fig. 9**)

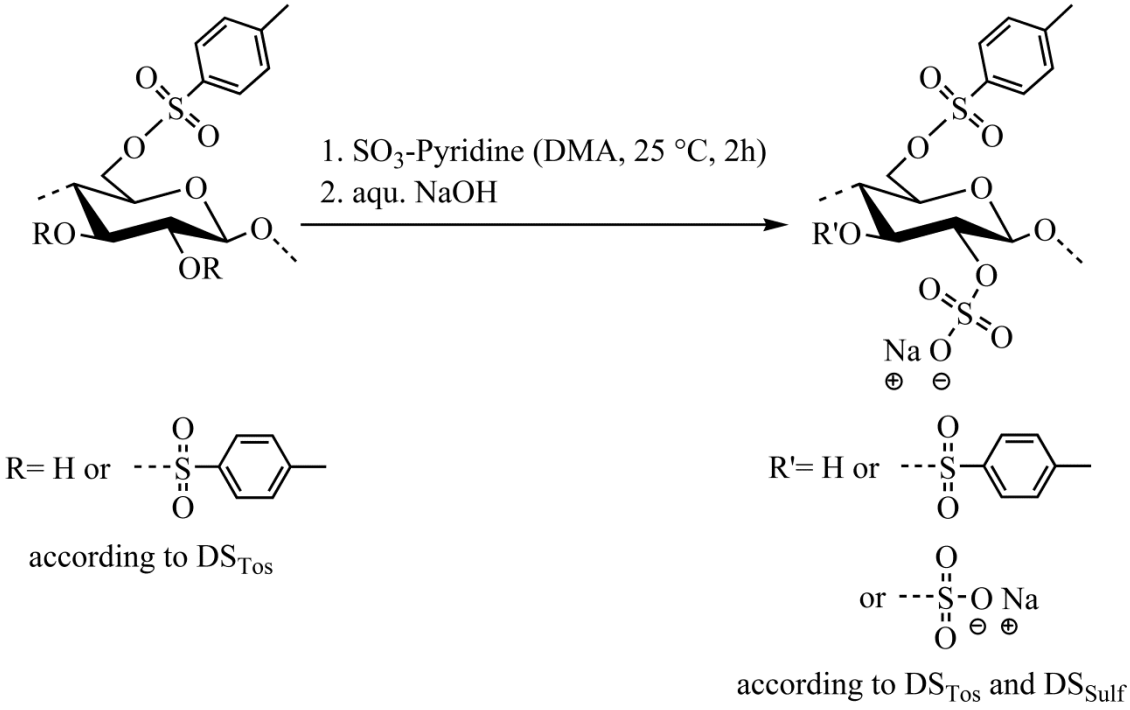

Fig. 9. The synthesis path for the molecular structure of tosyl cellulose sulfate. (adapted from Heinze and Rahn 1996).

Sulfonic acid esters of cellulose are prepared with various DS_{Tos} values varies from 0.46 to 2.02, which allows them to be soluble in DMAc for the subsequent esterification reaction. The tosylation takes place predominantly at position 6 of the repeating unit. A further prolongation of the reaction time does not lead to an increase in DS_{Sulf} (Heinze and Rahn 1996; Petzold-Welcke et al. 2009). The soluble sulfonic acid esters of cellulose in DMAc are used as starting compounds for sulfation of the remaining free hydroxyl groups. Thus, DS values of sulfate groups (DS_{Sulf}) in the resulting tosyl cellulose sulfates are between 0.34 and 0.80 (see **Table 4**). Tosyl groups, subsequently, act as a good leaving group for subsequent nucleophilic displacement reactions at position 6 (Heinze et al. 2006; Heinze and Rahn 1996).

Table 4. Conditions and results of the homogeneous sulfation of *p*-toluenesulfonyl (tosyl) cellulose with sulfur trioxide pyridine complex (SPC) in *N,N*-dimethylacetamide at room temperature. (adapted from Heinze and Rahn 1996).

Tosyl cellulose	Reaction conditions		Tosyl cellulose sulfate		
	Mol SPC/ Mol free OH-groups	Time in h		Solubility [c]	
DS_{Tos} [a]			DS_{Sulf} [b]	H_2O	DMSO
0.46	3.0	2.5	0.80	+	-
0.89	2.0	2.5	0.85	+	+
1.43	2.0	2.0	0.57	+	+
1.43	4.0	2.0	0.71	+	+
1.43	4.0	4.0	0.85	+	+
1.43	4.0	6.0	0.85	+	+
2.02	2.6	2.0	0.34	-	+

[a] Degree of substitution of tosyl groups calculated from S analysis; [b] Degree of substitution of sulfuric acid half ester groups calculated from S and Na analysis; [c] Determined at a concentration of 1 g/100 mL: + soluble, - insoluble, DMSO= dimethyl sulfoxide.

2.2.2. Introduction of amino functions into cellulose backbone by S_N reactions

Derivatization of cellulose by nucleophilic displacement (S_N) reactions is a suitable path for the preparation of novel highly engineered products (Heinze and Petzold-Welcke 2012). Thus, 6-amino-6-deoxycellulose and related derivatives were prepared from *p*-toluenesulfonyl (tosyl) cellulose by S_N reaction with azido groups at position 6. By subsequent reduction with $LiAlH_4$, the azido group is reduced to the amino moiety, and simultaneously, the remaining tosyl groups are completely removed (Baumann et al. 2003; Liu and Baumann 2002) (see **Fig. 10**).

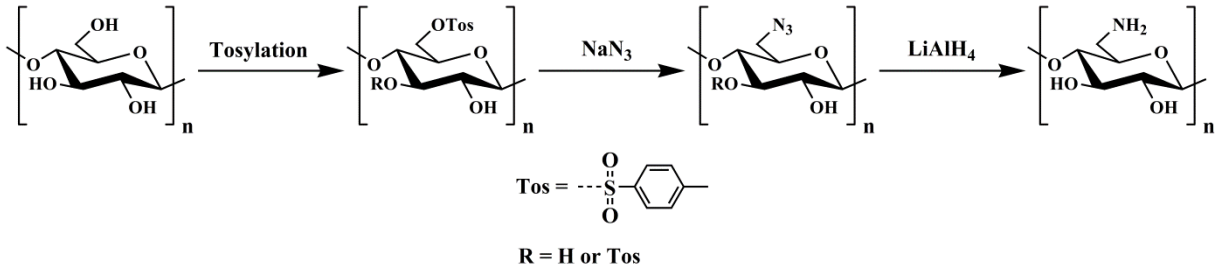

Fig. 10. The regioselective synthesis of 6-amino-6-deoxycellulose from *p*-toluenesulfonyl (tosyl) cellulose by S_N reaction with azido groups at position 6 using $LiAlH_4$. (adapted from Liu and Baumann 2002)

The path of tosylation, S_N with sodium azide and subsequent copper-catalyzed Huisgen reaction, has significantly broadened the structural diversity of polysaccharide derivatives because the method yields products that are not accessible via etherification and esterification, and the most commonly applied reactions are discussed in Liebert et al. 2006. The preparation of 6-deoxy-6-azido cellulose and subsequent copper-catalyzed Huisgen reaction of 1,4-disubstituted 1,2,3-triazols formed as linkers lead to novel cellulose derivatives with methylcarboxylate, 2-aniline, and 3-thiophene moieties. The 1,3-dipolar cycloaddition reaction of 6-azido-6-deoxycellulose with acetylenedicarboxylic acid dimethyl ester and subsequent saponification with aqueous NaOH yield bifunctional cellulose-based polyelectrolytes (Koschella et al. 2010). Moreover, water-soluble deoxy-azido cellulose derivatives (synthesized from tosyl cellulose) can be obtained by heterogeneous carboxymethylation applying 2-propanol/aqueous NaOH as a medium. Starting from the cellulose derivatives with different degrees of substitution (DS) of the azide moiety (0.58–1.01), various DS values of the carboxymethyl functions (1.01–1.35) were realized (Pohl et al. 2009). The carboxymethyl deoxy-azido cellulose provides a convenient starting material for the selective dendronization of cellulose via Huisgen reaction yielding water-soluble carboxymethyl 6-deoxy-(1-*N*-(1,2,3-triazolo)- 4-polyamidoamine) cellulose derivatives of first (DS 0.51), second (DS 0.44), and third generation (DS 0.39). A very

promising path to obtain amino group displaying polysaccharides is the S_N reaction of tosyl cellulose with di- or oligoamines leading to water soluble 6-deoxy-6-amino celluloses (Tiller et al. 1999; Berlin et al. 2000; Jung and Berlin 2005). Using the hydrodynamic technique of analytical ultracentrifugation, it was shown that the 6-deoxy-6-amino celluloses form multiple oligomeric species, and this protein-like behavior further substantiates the high potential of carbohydrates in nanoscience (Heinze et al. 2011).

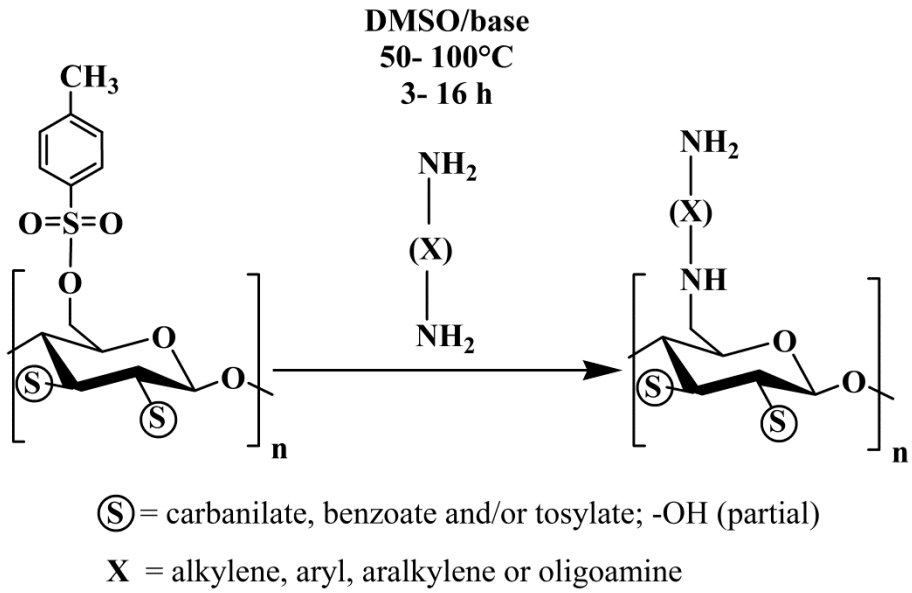

Fig. 11. Scheme of the conversion of 6(2)-O-tosylcelluloses or 6(2)-O-tosylcelluloses esters with aromatic and aliphatic diamino and oligoamino compounds. (adapted from Jung and Berlin 2005)

Moreover, 6-deoxy-6-amino celluloses were prepared with additional functional groups, such as acetate, benzoate, carbanilate, and methoxy at position 2 and 3 of the anhydroglucose unit (Berlin et al. 2003; Berlin et al. 2000; Jung and Berlin 2005; Tiller et al. 2001) (see **Fig. 11**).

2.3. Polyampholytes

Polyampholytes are macromolecules charged positively and negatively and carry both acidic and basic groups on their chains (Bekturov et al. 1990; Kudaibergenov 1999). Under appropriate conditions these groups dissociate in aqueous solution, producing ionic groups on the polymer chains and respective counter ions in solution. In case the groups are weak acids or bases, varying the pH of their aqueous solutions can change the net charge of polyampholytes. The isoelectric point is found at a specific pH value, when the numbers of positive and negative charges are equal. The net charge at the isoelectric point is zero, and the polyampholytes state is divided into polyacids and polybases. Thus, the polymers are nearly charge-balanced and exhibit unusual properties of polyampholytes. At pH values far above or far below the isoelectric point, the polymers show polyelectrolyte-like behavior (Dobrynin et al. 2004; Kudaibergenov 1999).

Ionic polymers can be divided into two groups: polyelectrolytes and polyzwitterions (e.g. polyampholytes or polybetaines) (Lowe and McCormick 2002). While polyelectrolytes are decorated either with cationic or anionic charges, polyampholytes carry both types of ionic groups within one macromolecule. Therefore, polyampholytes display unique physicochemical characteristics and have a huge potential for various applications, including protein separation and purification, binding and recovery of metal ions, and enhanced oil recovery (Kudaibergenov and Ciferri 2007). Some examples about the polyampholytes are denatured proteins (e.g., gelatin), proteins in their native state such as bovine serum albumin, and synthetic copolymers made of monomers with acidic and basic groups (Dobrynin et al. 2004). Polymers carrying ionic groups constitute different important classes of naturally-occurring biomacromolecules (e.g. polynucleotides or proteins) as well as different classes of synthetic polymers of commercial relevance (e.g. viscosifiers or flocculation agents). The theory of solution properties and other basic concepts regarding synthetic and natural polyzwitterions or polyampholytes were reviewed

by different authors (Lowe and McCormick 2002; Dobrynin et al. 2004; Ciferri and Kudaibergenov 2007).

2.3.1. Classification of polyampholytes (adapted from Kudaibergenov 1999)

Polyampholytes chains can include different types of zwitterions such as weak acid/weak base, strong acid/weak base, (or else weak acid/strong base) and strong acid/strong base monomers. Vinylpyridines and acrylic (or methacrylic) acid are typical examples of monomers incorporated in polyampholyte copolymers consisting of weak base and weak acid groups. While the copolymers of N-substituted allylamines and vinyl- or styrenesulfonic acids represent types of strong base/strong acid polyampholytes. The acidic and basic groups of polyampholytes can also be in salt forms with a low or high charge density along the macromolecules. From a macromolecular point of view (see **Fig. 12**), polyampholytes can be classified into random (a), alternating (b), graft (c), diblock (d) or triblock (e) sequences. In the case of polyampholytes with betaine-like structures (f), acidic and basic groups are located along the backbone of the polymer. In terms of classification of polyampholytes regarding their solubility, polyampholytes can be water soluble and water insoluble at pH values close to the isoelectric point (IEP). For example, over the whole interval of pH-values, unsaturated carboxylic acids and the equimolar copolymers of aminoalkyl(meth) acrylates are water soluble. By contrast, copolymers based on vinylpyridines and acrylic (methacrylic, vinyl- or styrenesulfonic) acid are insoluble at the IEP. Generally speaking, most of blockpolyampholytes possess wide insolubility regions. Hydrophobic polyampholytes represent the combination of the zwitterionic and hydrophobic structures in polyampholytes, which have behavior close to “polysoaps”.

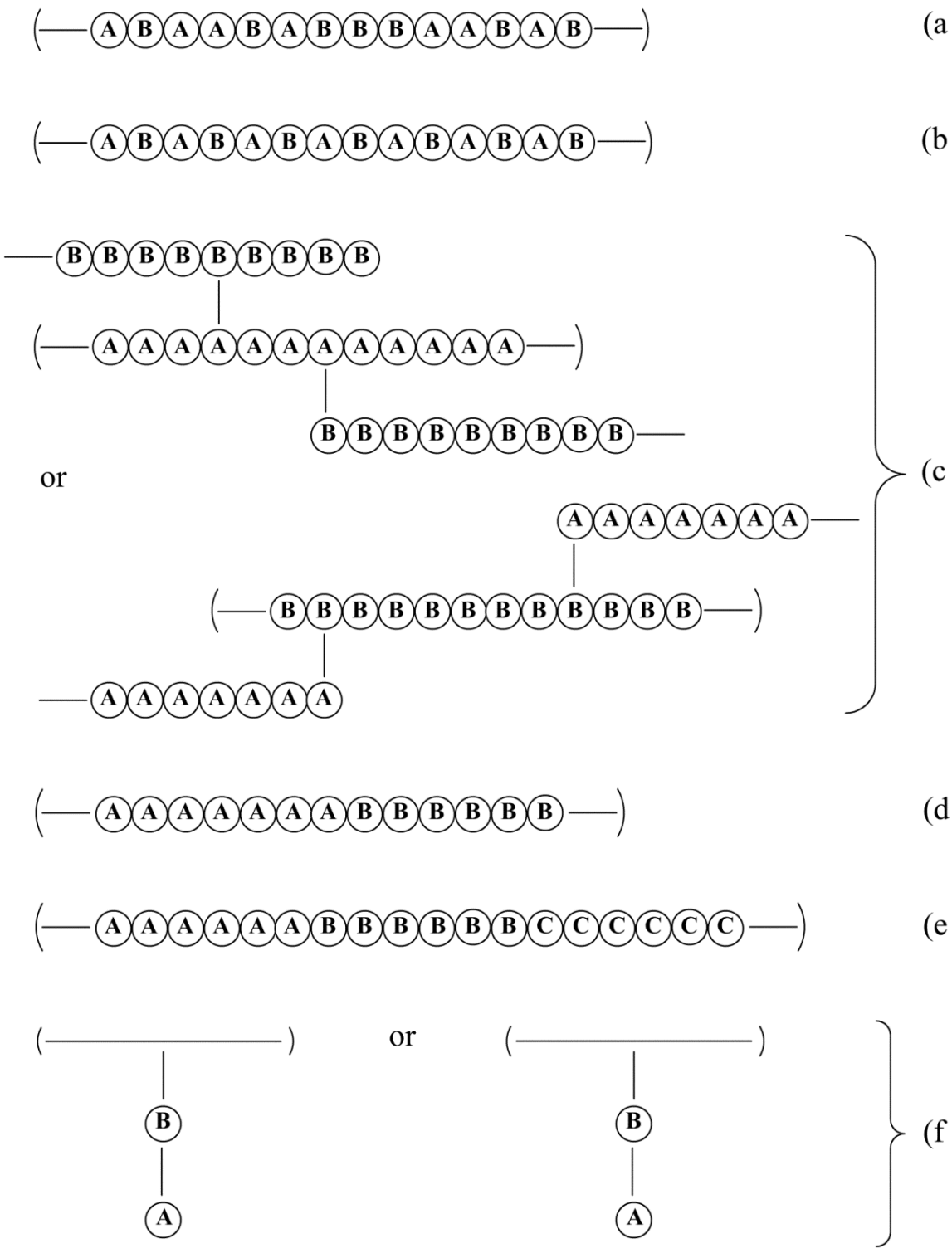

Fig. 12. Classification of polyampholytes dependent on the macrostructure; (a) random, (b) alternating, (c) graft, (d) diblock, (e) triblock sequence and (f) betaine structure. A, B and C are acidic, basic and neutral monomers, respectively. (adapted from Kudaibergenov 1999)

2.3.2. Acid-Base equilibrium in polyampholytes (adapted from Kudaibergenov 1999)

The nature of the functional groups and the microstructure of the chains of the polyampholytes determine the electrochemical properties. It is known in the literature that it is difficult to titrate the functional groups of polyampholytes in the proximity of the isoelectric point due to the fact that the acid-base properties of the amphoteric polyelectrolytes are different from the behavior of both polyacids and polybases (Tanford 1965). Only away from the isoelectric point, i.e. when negative or positive charges begin to be predominant, is the titration of acidic and basic groups straightforward. Then the acid-base equilibrium of polyampholytes can be analyzed using the modification of the Henderson–Hasselbach equation (Mazur et al. 1959; Merle and Merle 1982; Merle 1985; Merle 1987).

$$pH = \mathrm{p}K'_{\mathrm{a}} + log\,(\alpha\,/1 - \alpha)$$

$$pH = \mathrm{p}K'_{\mathrm{a}} + log\,(1 - \beta/\beta)$$

$\mathrm{p}K'_{\mathrm{a}}$ and $\mathrm{p}K'_{\mathrm{a}}$ are the acidic dissociation constants of acidic and basic groups, respectively, and α and β are the degree of ionization of acidic and basic groups of polyampholytes, respectively.

Katchalsky and Gillis (Katchalsky and Gillis 1949) have suggested a theoretical equation for determination of the dissociation constants of ionizing groups. The equation is based on the model of electrostatic potential smeared along the backbone of the polymer chain. It takes into account the electrostatic interaction between different chain segments as well as between ionic groups and low molecular weight electrolytes. The theoretical study was compared to the experimental and the agreement between theory and experimental is satisfactory.

2.3.3. Adsorption of synthetic polyampholytes on disperse particles (adapted from Kudaibergenov 1999)

Adsorption of charged polymers on the surfaces or disperse particles is one of the important questions of polymer physics. In the literature, several experiments on the interaction of

synthetic polyampholytes with disperse particles were perfumed (Blaakmeer 1990; Neyret et al. 1995; Dobrynin et al. 1999; Ozon et al. 2002). The adsorption of a single polyampholyte chain on a planar solid surface imitating the latex particles was studied theoretically by (Joanny 1994). The theoretical results obtained by (Joanny 1994) are in a good agreement with the experimental results performed on the interaction of synthetic polyampholytes with colloidal dispersions (Musabekov et al. 1998). According to the theoretical study of (Joanny 1994), polyampholyte solutions are capable of being adsorbed on latex particles even when the overall charge of the polyampholytes solution has the same sign as that of the latex particles. Two cases must be considered in the interaction between the functional groups of the polyampholytes; they are short-range (strongly screened) and long-range (unscreened). In the case of the short-range interactions, Joanny used the replica trick and a Hartree approximation and showed that the adsorption of the chain can occur even if the chain has the same net charge as the surface and if the interaction potential is larger than a critical value, i.e.:

$$V_c = log\frac{f}{g}$$

where V_c is the adsorption threshold, f is the fraction of monomers having the same charge as the surface and g is the fraction of monomers having the opposite charge as the surface. There is no adsorption threshold ($V_c < 0$), when the adsorbing chain possess an overall charge opposite to that of the surface. If the total charge is small, the value of V_c is small where $\frac{f}{g} \approx 1$. If the $f = g$, the adsorption threshold is zero and the chain adsorbs always in spite of absence of the average potential acting on the monomers. Furthermore, it is noted that the chain can adsorb when the interaction potential is large enough even if it is in average repelled by the surface $f > g$.

2.3.4. Cellulose-based ampholytic polymers

Ionic cellulose derivatives are known for a long time. For example, carboxymethylcellulose, up to now the most important ionic cellulose ether, was first prepared in 1918 and produced commercially since the early 1920s in Germany (Thielking and Schmidt 2006). In the context of the development of smart polymers by chemical modification of polysaccharides, one of the main research areas is the design and synthesis of ionic cellulose derivatives applying unconventional methods and reaction media for cellulose functionalization (Heinze 1998; Heinze and Liebert 2001; Liebert and Heinze 2001; Heinze and Koschella 2005; Gericke et al. 2009a). Several unexpected properties, e.g. the protein-like oligomerization of so called amino celluloses were investigated (Heinze et al. 2011), that substantiate the huge potential of ionic cellulose derivatives. Surprisingly, it can be noticed that although numerous ionic cellulose derivatives are known up to now the research on polyampholytes is mainly focused on synthetic polymers. Except for a few research articles, there are no results published, which are concerned with the direct functionalization of cellulose towards cellulosic polyampholytes. Zheng et al. 1995, 1996 synthesized an amphoteric cellulose derivative containing anionic carboxymethyl and cationic 2-hydroxy-3-(trimethylammonium) propyl substituents. The authors highlighted the advantages of cellulose based polyampholytes and their significance for both theoretical studies and practical applications. In particular, the possibility to adjust the charge densities per repeat unit and the types/distributions of substituents turned out to affect the properties in aqueous solutions. However, to the best of our knowledge, since this work only one additional paper was published dealing with zwitterionically modified cellulose derivatives. Gaweł et al. 2010 synthesized novel polyzwitterions by grafting sulfobetaine side chains onto hydroxypropylcellulose (HPC) and used them for the preparation of antiadhesive surfaces, which may find use in biomedical applications (see **Fig. 13**).

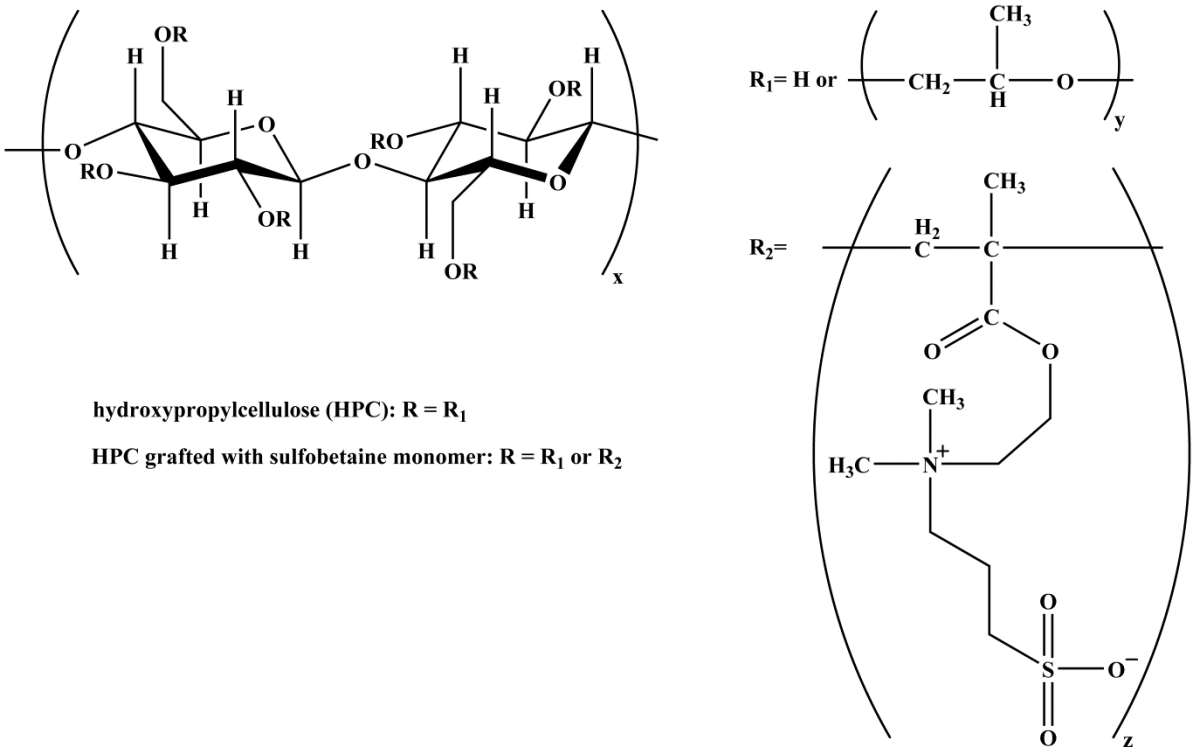

Fig. 13. Structures of hydroxypropylcellulose (HPC) and HPC grafted with sulfobetaine monomer; *N*-(3-sulfopropyl)-*N*-(methacryloxyethyl)-*N,N*-dimethylammonium betaine. (adapted from Gaweł et al. 2010)

2.3.5. Fibers with amphoteric character

It is known that purified (alkali pretreated) regenerated cellulose fibers are negatively charged due to the presence of carboxyl groups within their structures (Stana-Kleinschek and Ribitsch 1998; Stana-Kleinschek et al. 2002; Ramesh Kumar and Teli 2007; Stana-Kleinschek et al. 2001). While this implies that molecules containing cationic functionalities may be readily adsorbed on the fibers, providing their surfaces with amphoteric characteristics may further expand their potential usage by improving their surface properties and adsorbtivity, as well as their fiber dyeing capabilities. Although there are many published papers regarding the usage of different agents in order to functionalize fibers by introducing amphoteric characteristics, the use of polysaccharides due to their biodegradability and renewability is still a big challenge. There is

therefore a need to verify this possibility by conferring amphoteric characteristics to viscose fibers through adsorption of novel polysaccharide-based products. It has been reported in the literature that amphoteric polysaccharides, consisting of anhydrohexose units and modified by groups bearing at least one anionic and one cationic functional group, can be used for treating textile fiber articles. These amphoteric agents were used for preventing degradation, for enabling the protection of colors and for providing textile articles with crease-resistant and softening properties (Aubay et al. 2006). The amphoteric characteristics were introduced to the surfaces of the fibers by treating them with coupling agents like c-aminopropyl-triethoxy silane. The coupling agent was helpful by decreasing swelling and moisture uptake, in order to prepare the fibers for further specific processing steps (Bellmann et al. 2005). Amphoteric starch containing both cationic and anionic groups, i.e. 3 sulfopropyl groups/100 anhydroglucose units and 3.7 basic amino groups/100 anhydroglucose units, was used for sizing mixed fibers in the form of yarns, e.g., mixtures of polyester fibers and cotton fibers. In this way, the dyeing properties of the material were improved so that it was possible for the fibers to be dyed with acid dyes at a pH values below the isoelectric point and with basic dyes at a pH values above the isoelectric point (Elizer 1972). It was also found that the treatment of poly(hexamethylene adipamide) fiber flocks with an aqueous solution of an activator comprising an amphoteric compound containing both sodium sulfate and tertiary amino groups, enhanced the activity and the uniform dispersion to the flock within an electrostatic field without clumping (Hirshfield 1964). Cotton fibers treated with triethanolamine hydrochloride (TEOA-HCl) showed positive zeta potential values because the nitrogen present in the TEOA became protonated at acidic pH value. Thus, the dye uptake was improved due to the fact that the adsorption of the anionic dye on the cotton fibers was greater (Ramesh Kumar and Teli 2007).

3. Results and Discussion

3.1. Synthesis and characterization of aminocellulose sulfates

3.1.1. Synthesis

Polyampholytic cellulose derivatives were synthesized in three steps (**Fig. 14**). In the first step, the biopolymer **1** was allowed to react with *p*-toluenesulfonyl chloride in the presence of triethylamine in order to obtain tosyl cellulose (TC) **2a-d** (Rahn et al. 1996). The degree of substitution of tosyl groups (DS_{Tos}) was adjusted between 0.55 and 1.37 applying different molar ratios of *p*-toluenesulfonyl chloride to anhydroglucose units (AGU). The conversion of **2a-d** to tosyl cellulose sulfates (TCS, **3a-d**) with SO_3-pyridine complex was carried out homogeneously in N,N-dimethylacetamide (DMAc) (Heinze and Rahn 1996). The molar ratio of 1:5 (modified AGU: SPC) results in products with DS_{Sulf} values between 1.09 and 1.27 depending on the DS_{Tos} of starting TC (**Table 5**). The sulfonic acid half esters were converted into their sodium salts to avoid polymer degradation and hydrolysis of the functional groups.

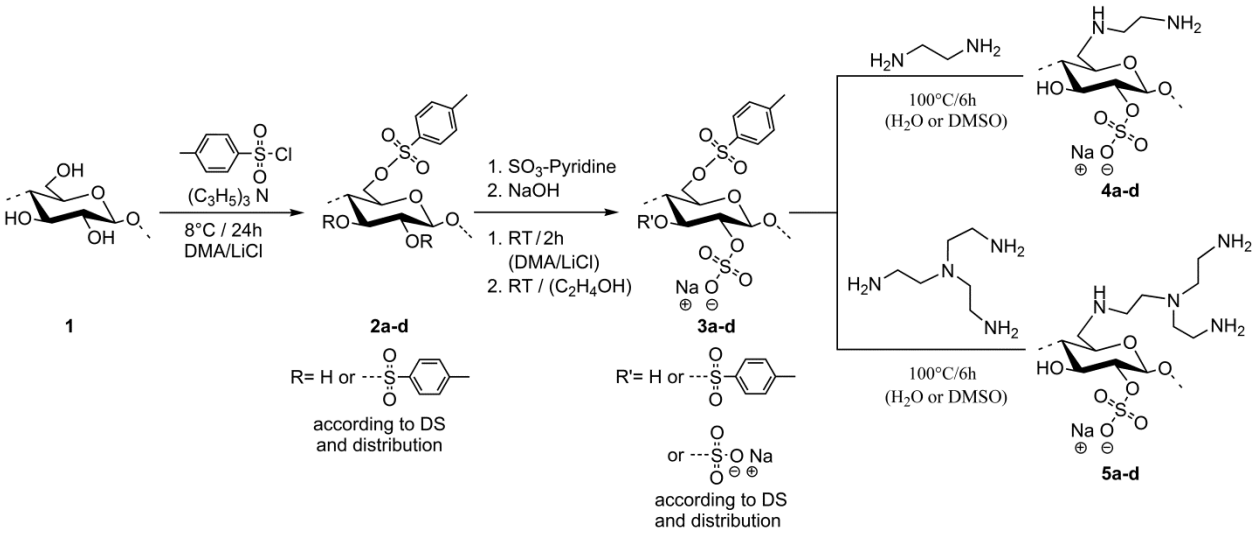

Fig. 14. Synthesis scheme for the preparation of 6-deoxy-6-(*ω*-aminoethyl)amino cellulose-2,3(6)-O-sulfate (**4a-d**) and 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (**5a-c**) via *p*-toluenesulfonyl (tosyl) cellulose (**2a-d**) and tosyl cellulose sulfate (**3a-d**).

As shown in **Table 5**, the DS_{Tos} of the TCS is slightly decreased compared to the DS of the initial TC. The obtained TCS were soluble in water and dimethyl sulfoxide (DMSO).

Table 5. Conditions for and results of the synthesis of tosyl cellulose (TC) and tosyl cellulose sulfates (TCS)

TC [a]					TCS [b]		
No	Molar ratio			DS [c]	No	DS [d]	
	AGU [e]	TEA	TosCl	Tos		Tos	SO_3
2a	1	1.6	0.8	0.55	**3a**	0.43	1.27
2b	1	2.6	1.3	0.85	**3b**	0.61	1.14
2c	1	3	1.5	0.95	**3c**	0.85	1.18
2d	1	4.0	2.0	1.37	**3d**	1.08	1.09

[a] Prepared by the reaction of cellulose with *p*-toluenesulfonyl chloride (TosCl) in the presence of triethylamine (TEA) as a base within 24 h at 8 °C in *N,N*-dimethylacetamide (DMAc)/LiCl; [b] prepared by the reaction of TC with sulfur trioxide pyridine complex (5 mol per mol of modified anhydroglucose unit) in DMAc as solvent; [c] degree of substitution, calculated on basis of the sulfur content determined by means of elemental analysis; [d] calculated on basis of the sulfur content and of UV-Vis spectroscopic measurements; [e] anhydroglucose unit.

It is well known that tosyl moieties are good leaving groups for nucleophilic displacement reactions (Heinze et al. 2006). However, in the case of TC and TCS only position 6 is accessible since the replacement of tosylate groups by weak nucleophiles (e.g. amines) follows the S_N2 reaction mechanism (Petzold-Welcke et al. 2009). The reaction of TCS **3a-d** with

multifunctional amines like 1,2-diaminoethane (DAE) or tris(2-aminoethyl) amine (TAEA) leads to amino cellulose sulfate (ACS) with different structures. Namely 6-deoxy-6-(*ω*-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS) **4a-d** and 6-deoxy-6-(2-(bis-N´,N´-(2-aminoethyl) aminoethyl)) amino cellulose-2,3(6)-O-sulfate (BAECS) **5a-d** were prepared under homogeneous reaction conditions in water or dimethyl sulfoxide at 100 °C.

Table 6. Conditions for and results of the synthesis of 6-deoxy-6-(*ω*-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS) and 6-deoxy-6-(2-(bis-N´,N´-(2-aminoethyl) aminoethyl)) amino cellulose-2,3(6)-O-sulfate (BAECS) starting from tosyl cellulose sulfates (TCS).

TCS			AECS [a]					BAECS [b]				
No.	DS		No.	DS [c]			WS [d] at pH	No.	DS [c]			WS [d] at pH
	Tos	SO_3		Tos	SO_3	AEA			Tos	SO_3	BAEA	
3a	0.43	1.27	**4a**	0	1.25	0.41	>5.8	**5a**	0	1.21	0.32	>9.0
3b	0.61	1.14	**4b**	0	1.14	0.58	>7.0	**5b**	0	1.12	0.45	>9.7
3c	0.85	1.18	**4c** [e]	0.09	1.41	0.85	>6.0	**5c** [e]	0.05	1.31	0.71	>9.7
3d	1.08	1.09	**4d**	0.19	1.08	0.86	>8.2	**5d**	0.21	0.70	0.74	>11.0

[a] Prepared by the reaction of TCS with 1,2-diaminoethane (25 mol per mol modified anhydroglucose unit) at 100 °C in H_2O within 6 h; [b] prepared by the reaction of TCS with tris(2-aminoethyl) amine (25 mol per mol modified anhydroglucose unit) at 100 °C in H_2O within 6 h; [c] degree of substitution, calculated on basis of the sulfur, nitrogen, and carbon content determined by means of elemental analysis; [d] water solubility; [e] prepared in dimethyl sulfoxide and the insoluble fraction (~5%) was removed.

To avoid intermolecular cross-linking, both amines (bifunctional DAE and trifunctional TAEA) were applied in molar excess of 25 equivalents (Tiller et al. 2001). The nucleophilic attack of the NH_2 groups to the tosyl functionalized C6 of the repeating unit led to the formation of C–NH linkage between the polymer and the amine. With increasing DS_{Tos} of the starting material the DS values of the corresponding amine (AECS and BAECS) increases (**Table 6**). Moreover, it can be noticed that no tosyl moieties remain in the ACS synthesized starting from TCS **3a** and **3b** with $DS_{Tos} < 1$. In case of TC **2c** with $DS_{Tos} \approx 1$, residues of tosyl groups can be detected in the final ACS (**4c** and **5c**, $DS_{Tos} \approx 0.05$ - 0.09). When using TCS **3d** $DS_{Tos} > 1$, a quantity of tosyl groups can be detected in the final ACS (**4d** and **5d**, $DS_{Tos} \approx 0.2$). The maximal DS_{BAEA} and DS_{AEA} achieved could not exceed 0.74 and 0.86, respectively. Obviously, a small amount of tosyl moieties are cleaved during reaction without replacement by amino groups. It is suggested in literature that the cleavage is driven by the basic character of the amine, which may act as catalyst for the reaction of the tosyl groups with impurities or the solvent (Bieser and Tiller 2011). In particular, carrying out the reaction in water or dimethyl sulfoxide this explanation holds true.

3.1.2. Characterization

The structure of the cellulose derivatives **2a-d**, **3a-d**, **4a-d**, and **5a-d** was studied by FT-IR- and ^{13}C-NMR spectroscopy as well as by size exclusion chromatography (SEC). **Fig. 15** shows the FT-IR spectra of cellulose **1**, TC **2a**, TCS **3a** and BAECS **5a**. The direct comparison of the spectra allows identifying the starting material, the intermediate products and the final ACS's. The FT-IR spectra of the cellulose starting material **1**, and the product formed upon its functionalization with tosyl groups **2a**, is clearly proofed by the typical signals in the spectrum of **2a**. More precisely, the signals arising from the $\nu_{CH_{arom}}$ (3063 cm^{-1}), $\nu_{C-C_{arom}}$ (1600 cm^{-1}), $\nu_{(as)SO_2(Tos)}$(1360 cm^{-1}), and $\nu_{(sy)SO_2(Tos)}$ (1176 cm^{-1}) are visible in the FT-IR spectrum of **2a**.

The subsequent modification with SO_3-pyridine complex generates sulfate moieties, which gave rise to the signals $\nu_{(as)SO_2(Sulf)}$ (1260 cm^{-1}) and $\nu_{(sy)SO_2(Sulf)}$ (1015 cm^{-1}) in the spectra of **3a**. Moreover, the signals arising from the tosyl groups (described above) are visible in the spectra of **3a** as well. The final S_N2 reaction of TCS **3a** with TAEA led to the formation of BAECS **5a**. As a consequence the signals arising from the tosyl groups disappear from the spectrum of **5a**. The new signals, which became visible, can be assigned to the ν_{NH_2}(3100 cm^{-1}) and δ_{NH_2}(1490 cm^{-1}) stretching.

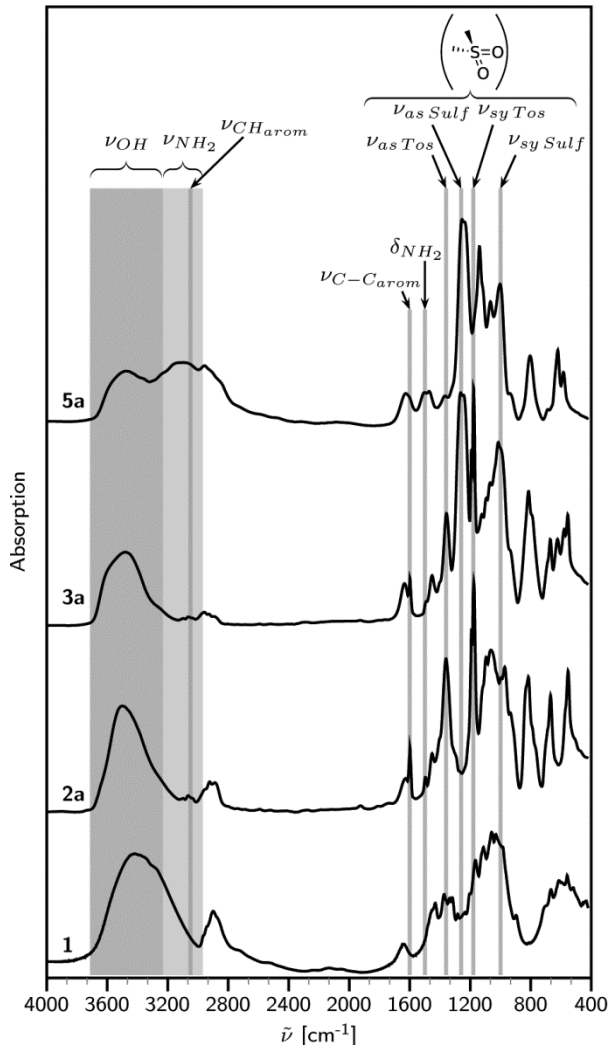

Fig. 15. IR spectra of cellulose (**1**), *p*-toluenesulfonyl (tosyl) cellulose (**2a**, DS_{Tos} = 0.55), tosyl cellulose sulfate (**3a**, DS_{Tos} = 0.43, DS_{Sulf} = 1.27), and 6-deoxy-6-(2-(bis-N´,N´-(2-aminoethyl)aminoethyl)) amino cellulose-2,3(6)-O-sulfate (**5a**, DS_{BAEA} = 0.32, DS_{Sulf} = 1.21).

Fig. 16 shows the ^{13}C-NMR spectra of cellulose **1** dissolved in DMSO-d_6/tetrabutylammonium fluoride (**Fig. 16a**), *p*-toluenesulfonyl (tosyl) cellulose (**2a**) dissolved in DMSO-d_6 (**Fig. 16b**), tosyl cellulose sulfate (**3a**) dissolved in D_2O (**Fig. 16c**), and 6-deoxy-6-(2-(bis-N´,N´-(2-aminoethyl) aminoethyl)) amino cellulose-2,3(6)-O-sulfate (**5a**) also dissolved in D_2O (**Fig. 16d**). All signals could be assigned on basis of literature data (Rahn et al. 1996; Heinze and Rahn 1996; Gericke et al. 2009a; Nikolajski et al. 2012). The spectrum in **Fig. 16b** reveals the success of the tosylation of cellulose. The ^{13}C resonances of the sp^2 carbon atoms of the aromatic residue of the tosyl group are visible in the range from 128 to 145 ppm. Moreover, the signal at 22 ppm can be assigned to the methyl group of the tosyl substituent. The direct comparison of the spectra of cellulose **1** and TC **2a** shows that the signals of C1-C5 are almost unaffected while a shift of C6 to lower field (C6-Tos) is visible after the esterification. Although it is not possible to give an exact measure from ^{13}C-NMR spectra as it is possible from the spectral integrals of proton NMR, the relative intensities of the signals of C6 and C6-Tos reveal that both species appear in almost same quantity. Keeping in mind that the DS of the sample measured is about 0.55 it can be concluded in accordance to literature that the tosylation takes place predominantly at position 6 of the repeating unit (Petzold-Welcke et al. 2009). The ^{13}C-NMR spectrum of TCS **3a** obtained by the conversion of TC **2a** with SO_3 pyridine complex (**Fig. 16c**) shows the same peaks assigned to tosyl substituent as described above. The sulfation is proven by the typical resonance of C6-O-SO_3^- at 66 ppm. Moreover, a larger line width could be noticed resulting from short lifetime of longitudinal and transversal magnetization. This typical phenomenon observed in NMR spectroscopy of polyelectrolytes is caused by the electrostatic repulsion forces and the resulting decrease in flexibility of the polymer chain. The spectrum in **Fig. 16d** was recorded after the reaction of TCS **3a** with TAEA. It is obvious that all resonances assigned to tosyl substituents disappeared. The new signals, which became visible at 37 and 52 ppm, can be assigned to the methylene carbon atoms of the BAEA substituents. Additionally the signal at 45

ppm, which results from C6-NH proofs the covalent linkage between the BAEA substituents and the cellulose backbone.

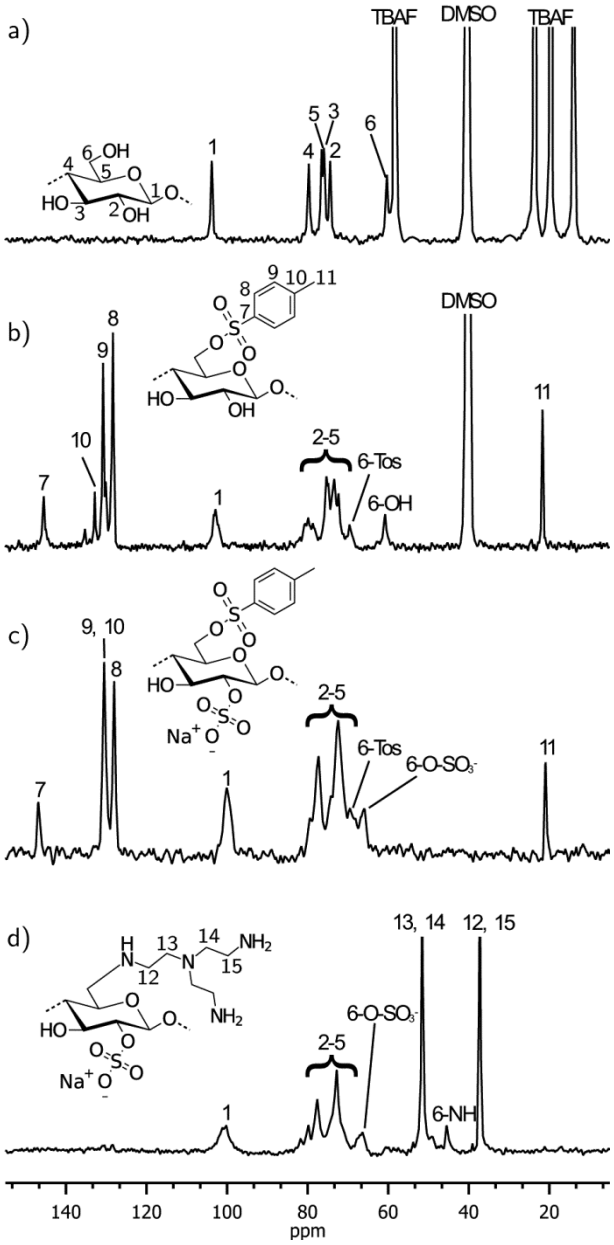

Fig. 16. ^{13}C NMR spectra of: a) cellulose (**1**) in DMSO-d_6 /Tetrabutylammonium fluoride (TBAF), b) *p*-toluenesulfonyl (tosyl) cellulose (**2a**, DS_{Tos} = 0.55) in DMSO-d_6, c) tosyl cellulose sulfate (**3a**, DS_{Tos} = 0.43, DS_{Sulf} = 1.27) in D_2O, and d) 6-deoxy-6-(2-(bis-N´,N´-(2-aminoethyl)aminoethyl)) amino cellulose-2,3(6)-O-sulfate (**5a**, DS_{BAEA} = 0.32, DS_{Sulf} = 1.21) in D_2O.

Preliminary experiments for the determination of the apparent molecular size of the ACS **4a-d** and **5a-d** were carried out via size exclusion chromatography (SEC). The M_W values obtained are 70,490 and 167,700 g mol^{-1} for AECS **4a** and BAECS **5a**, respectively. Considering the molar mass of the repeating unit, the corresponding DP_W values are 233 for **4a** and 536 for **5a**. In comparison with the DP_W of the initial cellulose (≈ 260-280), a slight decrease can be noticed in case of **4a** and an increase became apparent in case of **5a**, which is not possible. It is difficult to give a definite interpretation to the exact numbers since the different polymers might behave different at the SEC column and standard pullulan applied for calibration of the SEC system could be not representative for all polymers measured. However, the trend of an increasing molecular weight observed for BAECS might be attributed to an aggregation, resulting from the polyampholytic character of the ACS derivatives. The higher extent of aggregation and thus the higher molecular weight in case of BAECS result from the higher content of protonated amine groups present in the polymer (see below). An intermolecular crosslinking can be excluded, since the increase of molar mass and DP would be much more pronounced. Moreover, a cross-linking of polymers usually results in insolubility.

3.1.3. Properties

As it is typical for polyampholytes, the ACS **4a-d** and **5a-d** possess water solubility that depend on the pH value. The structure of the amine group carrying moiety and the DS of both, sulfate and amine groups, influence the solubility (**Table 6**). For example the BAECS **5a** (DS_{BAEA} = 0.32, DS_{Sulf} = 1.21) is well soluble at pH 11.5. A decrease of the pH value leads to the formation of colloids at pH ≈ 9 and a precipitation at pH < 9. By the subsequent increase of the pH value the polymer was dissolved again. This result can be easily explained from the zwitterionic behavior of the ACS polymers. The two primary amine groups of the BAEA substituents display a p*K*a value of about 10.8, the secondary amine groups located in the linkage to the cellulose backbone have a p*K*a value of 7.3, and the tertiary amine groups have a p*K*a value of 3.2 (Cakara

et al. 2003; Genco et al. 2012). It can be assumed that they are completely deprotonated at a pH value of 11.5 and the BAECS is negatively charged and water soluble due to the dissociated sulfate groups. Decreasing the pH value, results in the protonation of the amine groups and attractive interactions between positively charged ammonium groups and negatively charged sulfate groups progressively dominate the repulsive electrostatic interactions. In the vicinity of charge balance, the BAECS starts to precipitate due to intra- and intermolecular interactions. By increasing the pH value, the reverse effects take place and a solution of the polymer can be obtained again. In case sample **5b, 5c** and **5d** with a higher DS_{BAEA} and a lower DS_{Sulf} the pH value, which causes precipitation, is increased. Following the interpretation given above, this fact can be explained by the higher amount of ammonium groups at a certain pH value and the lower number of negative charges which need to be compensated to reach charge balance. The solubility behavior of the AECS **4a-d** was very similar to the behavior of the BAECS discussed above. The key difference being that the samples **4a-d** are generally soluble at lower pH values than **5a-d**. On one hand, this can be attributed to the different p*K*a values of the AEA substituent, which are about 9.0 for the primary and 4.5 for the secondary amine group (Zemljic et al. 2011). On the other, the total amount of amino group accessible to protonation is lower than in BAECS substituents, and therefore, a higher level of protonation is needed to reach charge balance which results in precipitation. However, the solubility in water under basic conditions and the trend of an increase of the pH value, which causes precipitation, with increasing DS_{AEA} and decreasing DS_{Sulf} was also observed as a general phenomenon.

3.2. Physicochemical properties and bioactivity of aminocellulose sulfates

6-deoxy-6-(*ω*-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS) and 6-deoxy-6-(2-(bis-N′,N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS) are completely water soluble at pH≥11 (**Table 6**). The physicochemical properties of AECS sample **4c** and

BAECS sample **5c** were studied as typical examples. The DS values of the functional groups in AECS **4c** are 0.85 for amino moiety (DS_{AEA}) and 1.41 for sulfate moiety (DS_{Sulf}). AECS **4c** possesses both primary and secondary amino groups. According to the structure DS x 2 gives a concentration of amino groups in AECS **4c** of 1.70 moles per AGU. In the case of BAECS **5c** there are four different amino groups (**5c**, DS_{Sulf} = 1.31, DS_{BAEA}= 0.71); two primary ones, a secondary one, and a tertiary amino group. Thus, from structural point of view, DS x 4 provides real concentrations of the amino groups, with a value of 2.84 moles per AGU.

3.2.1. Polyelectrolyte titration

The accessible amounts of sulfate groups of AECS **4c** and BAECS **5c** were determined by polyelectrolyte titration, and compared with the values of cellulose sulfate (CS). Thereby, facilitating a better understanding of the influence of amino group on the physicochemical behavior of the ACS derivatives is possible. AECS **4c**, BAECS **5c** and CS were titrated under the same conditions. The titration curves for AECS **4c** and BAECS **5c** show that with decreasing pH value of the ACS solutions, the negative charge arising from sulfate group, decreased proportionally (**Fig. 17a** and **Fig. 17b**). At pH≤ 5.38 and pH ≤ 9.65 for AECS **4c** and BAECS **5c**, respectively, the negative charge due to aggregation with the cationic ammonium groups could not be detected by polyelectrolyte titrations (**Table 7**). The titration curve of CS shows, as expected, that within the whole pH range from 11.5 to 3.0, the same average amount of 6.2 mMol g^{-1} was detected for the sulfate groups (**Fig. 17c**). This amount agrees well with the total amount of sulfate moieties of CS (6.57 mMol g^{-1}) obtained from EA. The amino groups in ACS derivatives decrease with decreasing pH value due to their interaction with the sulfate groups. Since the sulfate ion, SO_4^{2-}, is the conjugate base of the strong sulfuric acid, the sulfate moieties are deprotonated within almost the whole pH range**.** Acidification of the ACS solutions increases the amount of protonated amino groups. Thus, the possibility for electrostatic interaction

between both groups increases and the protonated amino and deprotonated sulfate groups are blocked.

Table 7. Amount of sulfate groups per gram of 6-deoxy-6-(*ω*-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS **4c**) and 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS **5c**) at different pH values obtained by polyelectrolyte titration.

AECS, 4c		BAECS, 5c	
pH	SO_3^{2-} [mMol g^{-1}]	pH	SO_3^{2-} [mMol g^{-1}]
11.7	3.86 ± 0.02	11.7	3.73 ± 0.07
10.8	3.38 ± 0.04	11.2	2.88 ± 0.02
10.2	2.77 ± 0.02	10.7	2.48 ± 0.02
8.5	1.51 ± 0.02	10.1	1.25 ± 0.11
7.3	0.65 ± 0.02	9.7	0.28 ± 0.02
5.4	0	8.9	0
3.6	0	7.2	0

Consequently, aggregation occurred, as indicated by the precipitation of the samples observed in the titration cell with decreasing pH value. Since there is no aggregation between the sulfate and amino groups at pH 11.7 (also proven by p*K* values determined via potentiometric titration as discussed below), the sulfate groups are fully accessible for detection by polyelectrolyte titration and, at this point, give the maximum values for the sulfate groups.

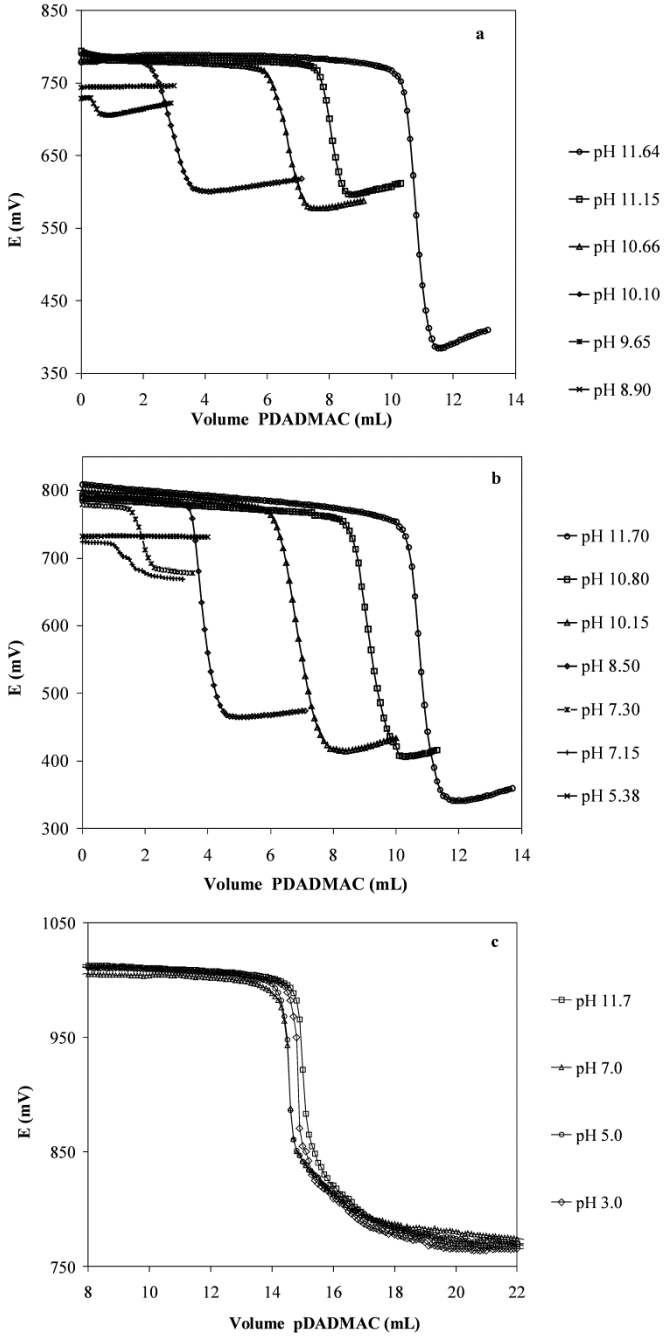

Fig. 17. Comparison of polyelectrolyte titration curves at different pH levels for three cellulosic derivatives in aqueous solution; a) 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS **5c**), b) 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS **4c**) and c) cellulose sulfate (CS). In every titration cell there are 3mg of dissolved polysaccharide, 39.8 ml of water adjusted to the desired pH value by means of NaOH/HCl 1M and 200 µl of Toluidine Blue which was used as the indicator. The titrant used was polydiallyldimethylammonium chloride (PDADMAC), c = 1 mM.

Thus, the amount of sulfate groups of AECS **4c** and BAECS **5c** at pH ~ 11.7, are 3.86 and 3.73 mMol g^{-1}, respectively, which agrees very well with the total sulfate amount obtained from EA; 3.97 mMol g^{-1} and 3.15 mMol g^{-1} for AECS **4c** and BAECS **5c**, respectively.

3.2.2. Potentiometric titration

The protonation behavior of the amino groups of AECS **4c** and BAECS **5c** was studied by potentiometric titration. The charging isotherms normalized to the mass of the sample indicate for AECS **4c** two protonation steps within the ranges 9.3 < pH < 0.8 and 5.7 < pH < 8.6 that correspond with the p*K* values determined of about 10.5 and 7.3. For BAECS **5c**, three protonation steps within the ranges 9.3 < pH < 10.8, 5.7 < pH < 8.6 and a roughly distinguished step within the range 2.7 < pH < 3.7 were found (**Fig. 18**).

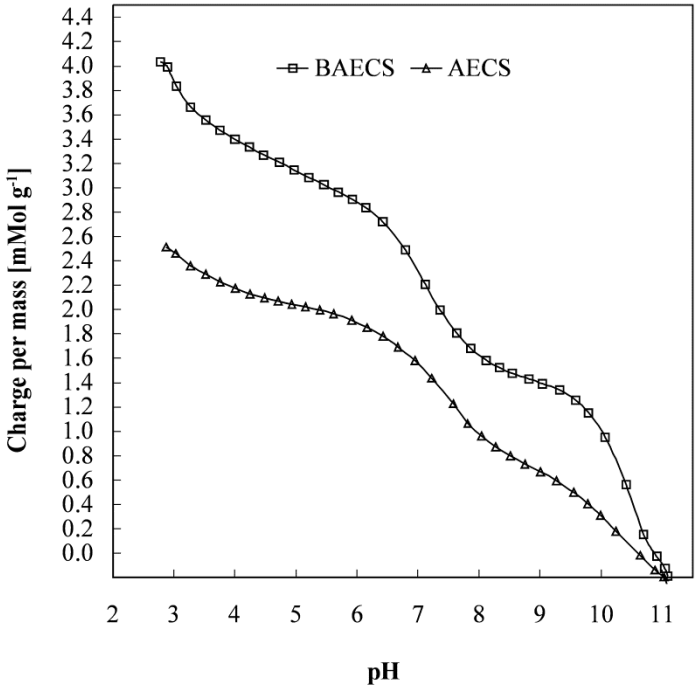

Fig. 18. The experimental charging isotherms resulted from potentiometric titration (back way) of 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS, **5c**) and 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS, **4c**) at 298 K.

Thus, from the charging isotherms, the p*K*a values of the products could be determined. Both primary amino groups have p*K*a values of around 10.5, whereas the secondary and tertiary amino groups have p*K*a values of about 7.3 and 3.2, respectively. This p*K*a values are in accordance with the literature values (Cakara et al. 2003). The protonation of brunched amino moieties first involves protonation of primary amine groups at the rim at high pH value, while the tertiary amine groups in the core protonate at lower pH value. The maximum positive charges detected from the charge isotherms shown in **Fig. 18** are 2.53 mMol g^{-1} for AECS **4c** and 4.07 mMol g^{-1} for BAECS **5c**. The p*K*a values were calculated using a non-linear least-squares fitting (Zemljic et al. 2011).

3.2.3. Conductometric titration

The conductometric titration curves of ACS, as presented in **Fig. 19**, possess two equivalence points (EP). The first EP represents the neutralization of the hydroxyl anions excess. The second EP represents the endpoint of the protonation reaction of the amino group. The titration curves end with a sharply rising slope due to the large conductivity of protons. The difference between the two equivalence points EP1 and EP2 corresponds to the volume of HCl needed to protonate the accessible amino groups. The results for conductometric titration, which indicated positive charges of 2.53 mMol g^{-1} for AECS **4c** and 4.02 for BAECS **5c**, agree very well with the maximum amounts of positive charges determined by potentiometric titration (**Table 8**). Thus, the results of conductometric titration supports those of potentiometric titration very well and support the high reliability of the results obtained.

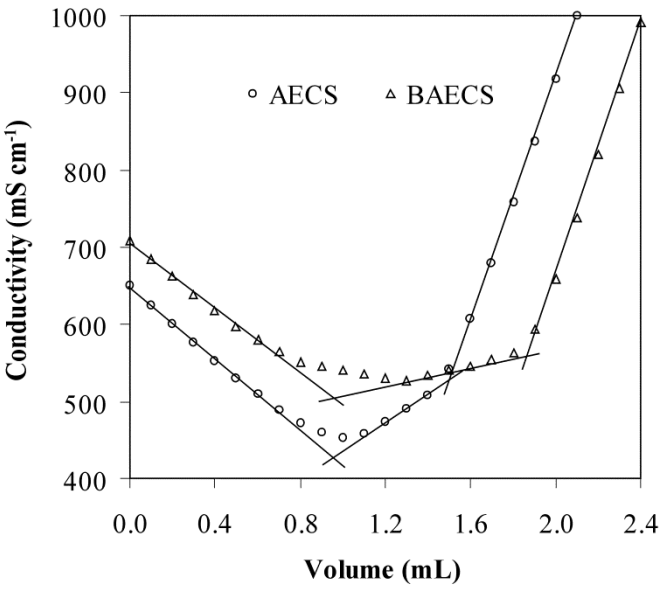

Fig. 19. Conductometric titration curve for amino cellulose sulfate samples 20 mg of 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS **4c**) and 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS **5c**) dissolved in 40 mL water containing 0.126 mL of 1M NaOH and titrated using 0.1 M aqueous HCl.

Table 8. Concentrations of the accessible amino groups obtained by potentiometric and conductometric titrations with the concentrations of total nitrogen in 6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS **4c**) and 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS **5c**) obtained by elemental analysis.

Sample Code	Accessible amount of NH_3^+ [mMol g^{-1}]		Total nitrogen [mMol g^{-1}]
	Conductometric titration	Potentiometric titration	Elemental analysis
AECS **4c**	2.53 ± 0.22	2.53 ± 0.20	4.79 ± 0.11
BAECS **5c**	4.02 ± 0.44	4.07 ± 0.04	7.24 ± 0.04

The total nitrogen content of the samples detected by EA accounts for 5.69 mMol g^{-1} for AECS **4c** and 7.54 mMol g^{-1} for BAECS **5c**. When these results are compared with the ones obtained from titrations, it is obvious that only about 44% to 53% of the total nitrogen was monitored by the titrations. This is expected due to the fact that with elemental analysis the total nitrogen presented in the samples is determined while with potentiometric and conductometric titrations only the accessible protonated amino groups are determined.

3.2.4. Zeta potential measurements

Zeta potential (ZP) measurements at different pH values were carried out in order to study the interactions of ACS particles formed by electrostatic aggregation, which are dispersed in the aqueous solution. The aggregation of dissolved ACS (with decreasing pH value) was proven via an investigation of the particle sizes by means of a light scattering particle size analyzer. It was found that the effective diameter of the ACS particles dispersed in the solutions increases from ~ 75 nm at pH 11.5 to several micrometers at pH ≤ 10 and pH ≤ 7 for both BAECS **5c** and AECS **4c** samples.

ZP versus pH, ζ=f(pH) plotted for AECS **4c** and BAECS **5c** (**Fig. 20a**) confirmed amphoteric characteristics for the ACS. AECS **4c** and BAECS **5c** behaved as ampholyte, which form zwitterions within a certain range of pH values. The ZP curve of BAECS **5c** in pH range between 9.7 to 11.5 shows that the product possesses negative charges. At pH 11.5, the ZP values were smaller than at pH value of 11.0. It is believed that the smallest size of particles occurs at pH 11.5. Within the pH range from 11.0 to 9.7, the negative values of ZP decreased dramatically. It is believed that the negative charge of the sulfate groups starts to be blocked with increasing number of protonated amino groups. At pH 9.7, the average charge of the aqueous solution is zero, which represents the isoelectric point (IEP) of the sample. It can be concluded that IEP for the BAECS **5c** solution is reached at this pH value, i.e. the total amount of sulfate

groups is fully aggregated with the amino groups. At pH ≤ 9.7, the zeta potential values indicate positive charges in the polymer solution. Due to the fact that total amount of amino groups in the samples is higher than the total amount of sulfate groups, the excess of the amino groups, which exceeds the equivalent of the sulfate groups, is protonated and has positive zeta potential values.

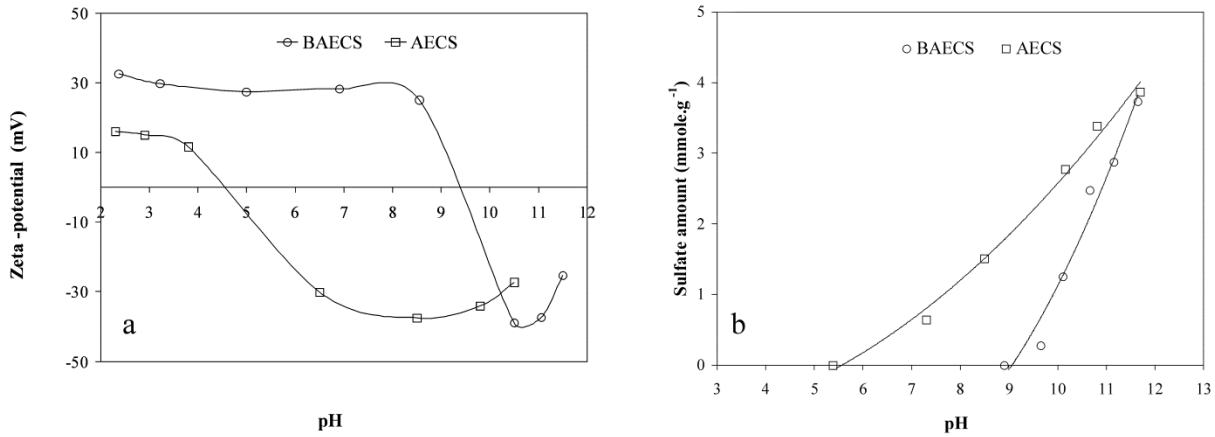

Fig. 20. a) Zeta potential as a function of pH value of the aqueous solution of 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS **4c**) and 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS **5c**), b) Negative charge isotherm representing the accessible amount of sulfate group determined by means of polyelectrolyte titration at different pH levels for AECS **4c** and BAECS **5c** aqueous solutions.

AECS **4c** generally behaves comparably to BAECS **5c**, which can be seen from the ZP curve with the difference that the IEP shifts to pH 4.7. The considerable contribution of the secondary amino groups during aggregation with the sulfate groups shifts the isoelectric point to the acidic area, on one hand. On the other hand, since the amino moiety of the BAECS **5c** possesses double the amount of primary amino groups of AECS **4c**, the isoelectric point of BAECS **5c** sample is shifted to the vicinity of the p*K*a value for the primary amino group (**Fig. 20a**). The results obtained from ZP measurements were compared with the results of polyelectrolyte titrations (**Fig. 20b**). It can be seen that at pH ~ 9.2 for BAECS **5c** and ~ 5.4 for AECS **4c**, the negative

charge of the sample is absent, which agrees very well with the values for IEP obtained using the ZP evaluations.

3.2.5. Overview of the physicochemical characteristics of the ACS derivatives

In the light of the aforementioned techniques, the physicochemical behavior of ACS derivatives can be summarized. At pH 11.5, the products are negatively charged and give the highest detected amounts of sulfate groups. At this stage, the amino groups are fully deprotonated (**Fig. 21a**). By decreasing the pH value of the medium, primary amino groups will start to protonate in accordance with their p*K*a value of ~ 10.5. Due to the assumption that intra- and inter-chain electrostatic attractions in the polyampholyte may cause aggregation (Rinaudo 2006; Roy et al. 2006), protonation of the amino groups creates cations, which may lead to aggregation with the negatively charged sulfate groups. When the protonation process produces an equivalent amount of positive charges equal to the total negative charges existing in the ACS solutions, the isoelectric point is reached and the products starts to aggregate, forming colloidal particles. At the isoelectric point, it is assumed that the total amount of sulfate groups in AECS **4c** and BAECS **5c** samples is countered with the available protonated amino groups. Thus, the products solutions remain non-charged at this stage (**Fig. 21b**)**.** Since the amounts of amino groups are higher than the amounts of sulfate groups in both AECS **4c** and BAECS **5c**, access of protonated amino groups at pH values < IEP yields positively charged solutions (**Fig. 21c**).

3.2.6. Bioactivity

It has been realized that by changing the pH value, the accessibility and protonation/deprotonation of amino and sulfate groups may be manipulated. Due to the presence of both groups responsible for specific bioactive properties (as discussed in the Introduction), it

was hypothesized that by manipulating the amount of accessible positive or negative charges of ACS, the bioactive properties of samples can also be manipulated.

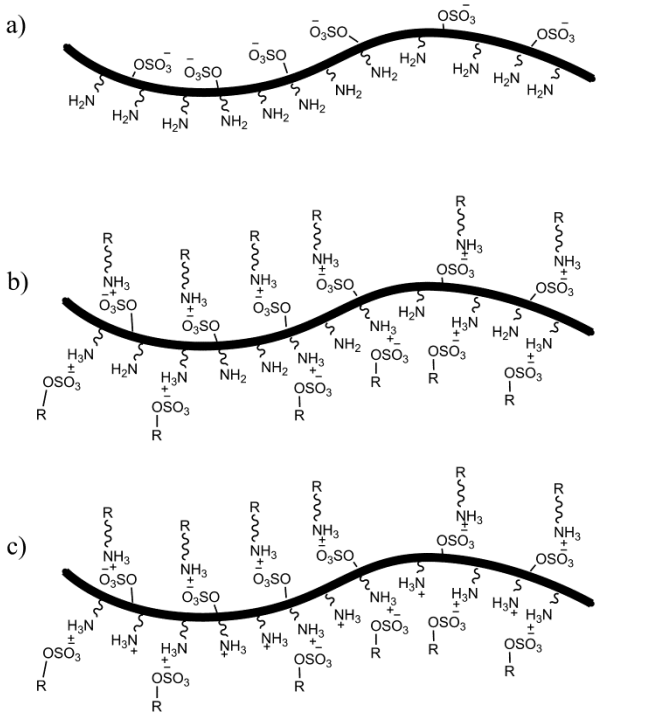

Fig. 21. Physicochemical behavior representative scheme of amino cellulose sulfate derivatives; a) at pH>11 amino groups are fully deprotonated and ACS polymers are negatively charged, b) The polymer is in the isoelectric point state at pH ~9.7 for BAECS **5c** and at ~4.7 for AECS **4c**, c) Amino groups are fully protonated at pH<3 and ACS polymers are positively charged.

3.2.6.1. Antimicrobial activity

Antimicrobial activity is mostly driven by ammonium groups. The higher the number of ammonium groups, the higher the antimicrobial activity (Másson et al. 2008). As a definition, the antimicrobial reduction (determined by standard ASTM E2149-01) of any product should be greater than 75 % in order to be considered as an antimicrobial agent (Kim et al. 1998; Seo et al.

1991). For cellulose sulfate (0.01 wt.%) solution, it was found that the antimicrobial activity for the studied pathogens is reduced to less than 60%, i.e. according to the definition CS shows insufficient antimicrobial activity. However, no negative sulfate groups exist at pH values lower than the detected isoelectric points of ACS (**Fig. 21b**). In other words, the ACS solutions at pH ≤ 9.7 for BAECS **5c** and pH ≤ 4.7 for AECS **4c** possess only positive charges arising from excess of protonated ammonium groups (**Fig. 21c**).

The antimicrobial test for dissolved ACS samples (0.01 wt. %) was performed *in vitro* by measuring the reduction in growth of typical pathogenic microorganisms: *Staphylococcus aureus* as gram positive bacteria, *Escherichia coli* as gram negative bacteria and *Candida glabrata* as a fungus. Since the pH values of the skin surface and most of the physiological fluids of the human body range from 4.5 to 8.0 (Jolly et al. 1961; Levin and Maibach 2008; Pang et al. 2007; Yosipovitch et al. 1993), the antimicrobial test of ACS was performed at standard pH values of 5 and 6.8. It was noticed that the inhibition for the tested pathogenic microorganisms was higher at pH 5 compared to pH 6.8 (**Fig. 22**). This was expected due to the fact that with the lowering of pH value the amount of protonated ammonium groups increased. Moreover, this confirms the relationship between the antimicrobial activity and the content of protonated ammonium groups. The antimicrobial test of the samples showed an increment of the reduction for *Staphylococcus aureus* from ~50% to ~90% in the case of AECS **4c** and from ~65% to 85% for BAECS **4c** with decreasing the pH values from 6.8 to 5. Thus, the decrease of the pH value of the sample leading to an increase of the amount of amonunium groups, introduces antimicrobial activity of samples regarding *Staphylococcus aureus*. However, a decrease of the pH value did not yield a reduction the growth of Gram negative *Escherichia coli*. At both pH values, the reduction of *Escherichia coli* was below 75% and thus the samples are inactive for this pathogen inhibition. Due to the complex structure of the cell wall of Gram negative bacteria, the inhibition it is more difficult compared to Gram positive bacteria. Comparable slight inhibition for *Escherichia coli* was observed for chitosan (Fras Zemljic et al. 2011; Zheng and Zhu 2003). Since the reasons for the

antimicrobial character of chitosan is the ammonium group (Shahidi et al. 1999) it is believed that the mechanisms for the antimicrobial activity of ACS and chitosan is similar for both Gram positive and Gram negative bacteria.

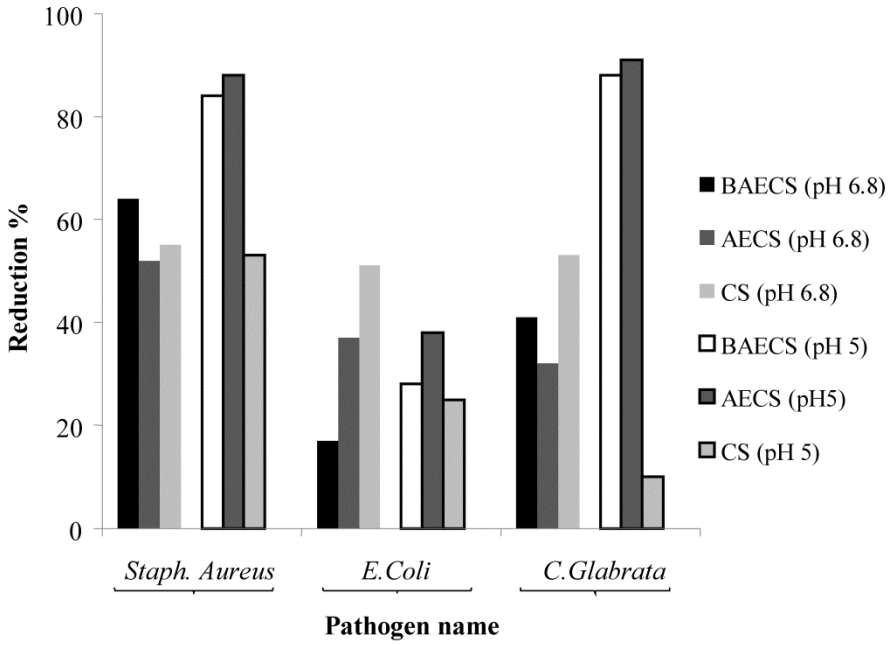

Fig. 22. Antimicrobial activity of the solutions of 6-deoxy-6-(2-(bis(2-aminoethyl)aminoethyl-amino) cellulose sulfate **(**BAECS **5c**), 6-deoxy-6-(2-aminoethyl) cellulose sulfate **(**AECS **4c**) and cellulose sulfate CS at pH 6.8 and pH 5. The Antimicrobial activity was expressed as R=% reduction of the organisms after contact with the test specimen compared to the number of bacterial cells surviving after contact with the control.

The antifungal test for AECS **4c** and BAECS **5c** showed that with decreasing pH value from 6.8 to 5 the reduction of *Candida glabrata* increases significanly from ~ 35 % to ~ 90 % (**Fig. 22**). It has been proven that the protonation of amino groups (increasing by decreasing of pH value) significantly influences antimicrobial activity of ACS. Both ACS samples inhibited Gram positive bacteria *Staphylococcus aureus* and pathogen fungus *Candida glabrata* at pH 5, where

most of the amino groups are expected to be protonated. The ACS samples do not cause a reduction of *Escheria coli* at this pH value as discussed above.

3.2.6.2. Antitrombogenetic activity

Since sulfate groups in polysaccharides may impart anticoagulant activity (Robert 1955; Doctor et al. 1991; Groth and Wagenknecht 2001), the antithrombogenicity for ACS was evaluated *in vitro* by measuring the APTT values of the dissolved samples. APTT was plotted as a function of the samples concentrations (**Fig. 23**). The test for BAECS **5c** solutions at pH ~ 8 showed a prolongation of the APTT when the concentrations of the sample exceeded 100μ mL^{-1}.

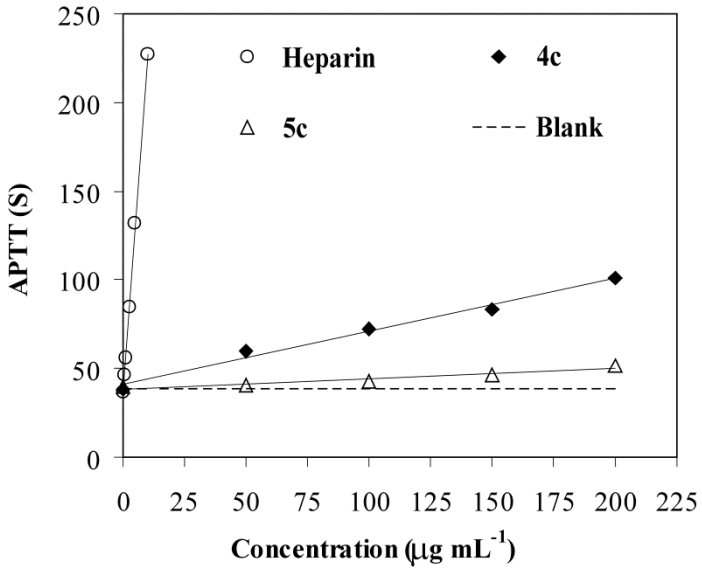

Fig. 23. APTT of 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS **4c**), 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS **5c**) and heparin as a function of polysaccharide concentration.

In the case of AECS **4c**, the APTT of the sample is prolonged when the concentrations are equal or exceed 50μ mL^{-1}. According to the isoelectric points of the samples, in contrast with BAECS **5c**, the accessible amounts of sulfate groups in AECS **4c** are still detectable at pH 8. Therefore, the antithrombogentic activity of AECS **4c** is higher compared to BAECS **5c**. In spite of this,

both samples prolong the APTT but in comparison with heparin, which prolongs APTT significantly, even at concentrations lower than 1 μ mL^{-1} (Hirsh and Raschke 2004; Whitfield and Levy 1980), they have only minor anticoagulation activity.

In order to more deeply understand the influence of DS values of sulfate and amino moieties on the APTT prolongation, the samples AECS **4a-4d** which have different DS_{AEA} and DS_{Sulf} values were studied (**Fig. 24**).

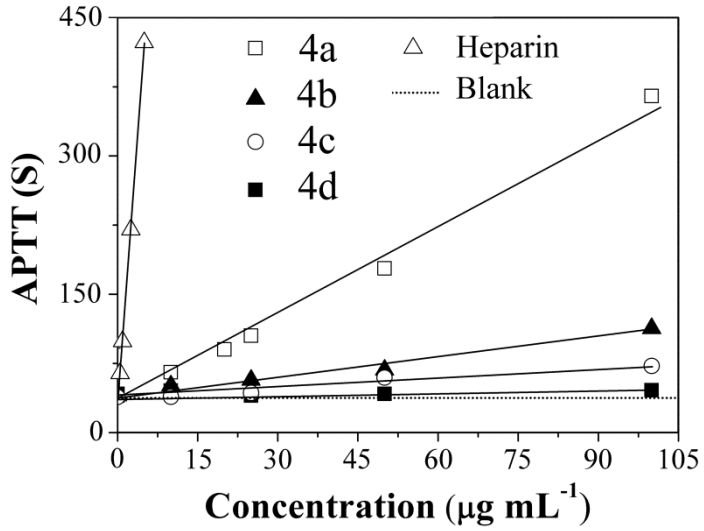

Fig. 24. APTT of 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS **4a-4d**) and heparin as a function of polysaccharide concentration.

The antithrombogentic activity of AECS (**4a**, DS_{Sulf} = 1.25, DS_{AEA} = 0.41) was the highest compared to the other samples AECS **5b - 4d**. The reason is that the sample **4a** possesses the lowest DS_{AEA} value compared to the other samples. Thus, the lower the aggregation between amino and sulfate groups, the higher the amount of sulfate groups which contribute to the prolongation of APTT values. The antithrombogentic activity of the sample (**4b**, DS_{Sulf} = 1.14, DS_{AEA} = 0.58) was lower than in the case of **4a** due to increment in DS_{AEA} and decrement in DS_{Sulf} compared to the sample **4a**. Therefore, higher aggregation levels than in the sample with **4a** occurred, decreasing the APTT prolongation of **4b**. The sample (**4c**, DS_{Sulf} = 1.41, DS_{AEA}=

0.85) possesses the highest DS_{Sulf} value compared to the other samples, but the majority of sulfate groups were aggregated with amino groups due to the excess of amino moiety which lowered the APTT prolongation. The same discussion as in the case of **4c** can be used for the sample (**4d**, DS_{Sulf} = 1.08, DS_{AEA} = 0.86) with a difference that the DS_{Sulf} in **4d** is lower than the in the case of **4c** which caused more aggregation between amino and sulfate groups than in the case of **4c** and the sample caused a slight prolongation in APTT compared to the blank.

3.3. Characterization of viscose fibers modified with aminocellulose sulfates

3.3.1. Efficiency of coating viscose fibers with amino cellulose sulfates

Solution of BAECS and AECS (here denoted just ACS) were prepared at pH 11, with the aim of fully deprotonating all of the amino groups. Thus, precipitation due to electrostatic interaction between the amino and sulfate groups within the solution could be avoided, as shown in our previous publication (Genco et al. 2012). The equilibrium desorbed amounts in acidic medium detected by TN were 34% and 39% for AECS-Vis and BAECS-Vis, respectively, thereby giving an indication of the adsorbed amino cellulose sulfate's stability (more than 60 % for both samples) on the viscose fiber.

3.3.2. XPS measurements

The surface elemental content of the non-coated and coated viscose fibers 269 determined by XPS is shown in **Table 9**. The amount of nitrogen on BAECS-Vis was 2.2 atom %, which is around 5 times higher than the amount of nitrogen on the AECS-Vis (0.43 %). This is higher than expected just from the fact that every amino moiety of BAECS contains 4 nitrogen atoms while the amino moiety of AECS contains only 2 nitrogen atoms. A 5 times higher concentration of nitrogen in BAECS-Vis in comparison with the AECS-Vis nitrogen concentration may also be explained as being due to the higher molecular weight and greater degree of branching of BAECS in comparison with AECS. Thus, the adsorption of BAECS to the viscose fibers may

only be restricted to a limited surface domain, whilst the smaller and more linear AECS macromolecule may also penetrate into the viscose fiber pores (inner fiber parts). Sulfur was also detected on the surfaces of the coated fibers (0.80% and 0.45% on BAECS-Vis and AECS-Vis, respectively). These differences support the notion that AECS-Vis may penetrate into the fibers while BAECS-Vis remains on the surface.

Table 9. Surface chemical composition of the non-coated fibers (B-Vis), the coated fibers with 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS-Vis) and the coated fibers with 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS-Vis), as measured by X-ray photoelectron spectroscopy (XPS).

Sample	C%	N%	O%	S%
B-Vis	64.1	0	35.9	0
AECS-Vis	64.9	2.20	31.5	0.80
BAECS-Vis	61.5	0.43	36.3	0.45

3.3.3. Potentiometric titration

The protonation behaviors of the existing moieties on B-Vis, AECS-Vis, and BAECS-Vis were studied using potentiometric titration. The charging-isotherms normalized to the mass of the fibers (**Fig. 25**) exhibited a negative charge for the non-coated fibers B-Vis with a plateau at ca. -25 mMol kg^{-1}, which is present at due to the presence of carboxyl groups (Stana-Kleinschek et al. 2002). At low pH values the charges of BAECS-Vis and AECS-Vis were positive due to protonation of the amino groups. The maximum positive charge detected from the charging isotherms of the coated fibers AECS-Vis and BAECS-Vis was 7.4 and 14.1 mMol kg^{-1}, respectively, with charge reversal (point of zero charge, PZC) that occurred at pH = 4.0 for BAECS-Vis and pH = 3.4 for AECS-Vis. The indicated approximate p*K*a values ~3.40, ~3.75,

and ~4.00 of the studied fibers B-Vis, BAECS-Vis, and AECS-Vis, respectively, were obtained according to the method described in the literature (Cakara et al. 2009). It was obvious that the p*K*a values in the samples BAECS-Vis and AECS-Vis were higher than the p*K*a value of B-Vis. This was due to the fact that the amino groups of ACS, on the coated fibers, possessed p*K*a values higher than the p*K*a value of the carboxyl groups existing on the non-coated fibers B-Vis (Genco et al. 2012; Fras et al. 2004). However these values did not approach the p*K*a value of the amino groups regarding the ACS product; due to the fact that a higher amount of acidic groups in comparison with amino groups (see **Fig. 25**) was present in the fiber. The anionic groups exceeded the cationic ones; the latter is also the reason for such a low PZC.

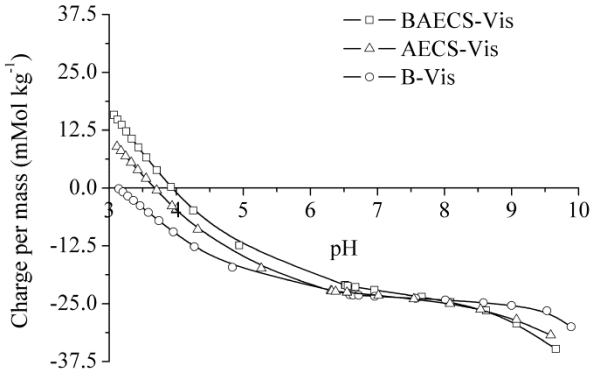

Fig. 25. The experimental charging isotherms, normalized to the mass of the fibers, resulted from potentiometric titration (front way) of the non-coated fibers (B-Vis), the coated fibers with 6-deoxy-6-(2-(bis-N´, N´-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS-Vis) and the coated fibers with 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS-Vis) at 298 K.

3.3.4. Conductometric titration

The titration curves of BAECS-Vis, AECS-Vis and B-Vis (**Fig. 26**) show two equivalence points (EP). The first EP represents the neutralization of free hydrogen ions. The second EP represents the endpoint of the dissociation of weak acids (amino and carboxyl groups). The titration curves

end with a sharply rising slope due to the large conductivity of the hydroxyl ions. The difference between the two equivalence points EP1 and EP2 corresponds to the deprotonation of the accessible amino and carboxyl groups existing on the viscose fibers (Fras et al. 2004).

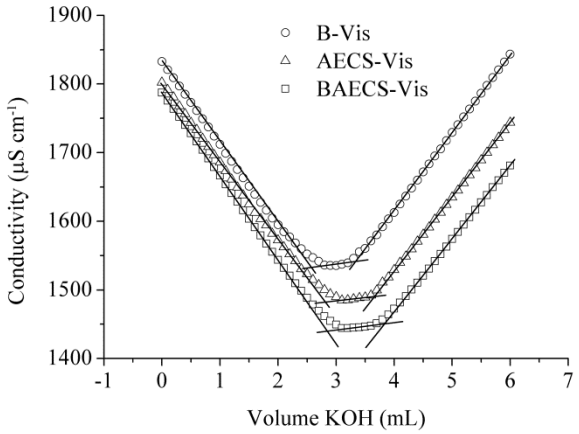

Fig. 26. Conductometric titration curve for the non-coated fibers (B-Vis), the coated fibers with 6-deoxy-6-(2-(bis-N´, N´-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS-Vis), and the coated fibers with 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS-Vis).

The accessible weak acids i.e. sum of the carboxyl and amino groups determined by conductometric titration on B-Vis, BAECS-Vis, and AECS-Vis are 35.6, 52.9 and 43.7 mMol kg^{-1}, respectively **(Table 10).**

The charge detected on the non-coated fibers is due to the carboxyl groups only, while the charges detected on the fibers coated with amino cellulose sulfate are due to both the initial fiber carboxyl groups and the introduced protonated amino groups. The increase in the total amount of weak acids after ACS-adsorption onto fibers clearly shows the introduction of amino groups as already pointed out by potentiometric titration. Since the BAECS-Vis and AECS-Vis have the same initial content of carboxyl groups as the reference sample B-Vis, the increase of the charge

detected in the samples BAECS-Vis and AECS-Vis makes it possible to calculate the accessible amounts of amino groups on the coated fibers. The amount in BAECS-Vis (17.4 mMol kg-1) was nearly twice as high as the amount in AECS-Vis (8.1 mMol kg^{-1}) **(Table 10)**.

Table 10. Accessible amounts of amino groups determined by conductometric- and potentiometric titrations on the non-coated fibers (B-Vis), coated fibers with 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS-Vis), and coated fibers with 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS-Vis), if no electrostatic interaction between carboxyl and amino groups is predicted.

Sample	Conductometric titration		Potentiometric titration
	Total charge [mMol kg^{-1}]	Amino group charge [mMol kg^{-1}]	Amino group charge [mMol kg^{-1}]
B-Vis	35.6 ± 3.6	0	0
AECS-Vis	43.7 ± 4.2	8.1 ± 0.6	7.4 ± 0.5
BAECS-Vis	52.9 ± 4.7	17.4 ± 1.1	14.1 ± 0.9

This is due to the fact that the tris(2-aminoethyl)amine moiety in BAECS-Vis possessed twice as many 1,2-diaminoethane groups as AECS-Vis. The detected amounts of positive charge agree with the amounts of positive charge determined by potentiometric titration (see **Fig. 27**), which shows a linear relationship between the fiber charge values obtained from the conductometric titrations and those obtained from the potentiometric titrations. This demonstrates the high reliability for the results obtained by both methods and supports the notion that there is only

physical interaction between the ACS and viscose fibers. This has also been shown by other previous researchers (Cakara et al. 2009; Myllytie et al. 2009).

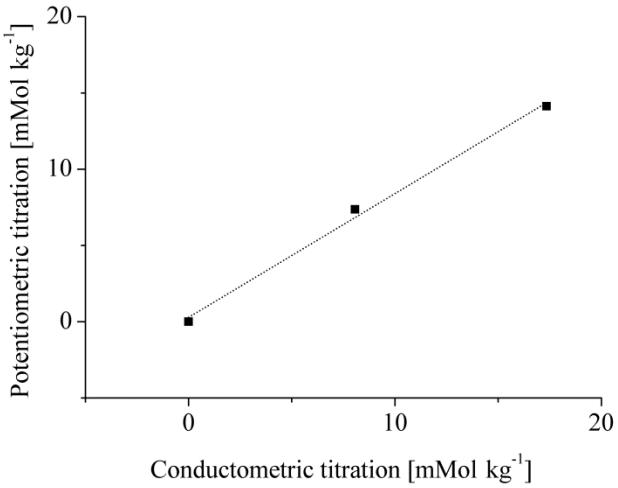

Fig. 27. Correlation between the fiber-charge values obtained from potentiometric and conductometric titrations.

3.3.5. Polyelectrolyte titration

A well-defined linear part of the isotherm was obtained from the polyelectrolyte adsorption on viscose fibers B-Vis, BAECS-Vis and AECS-Vis as shown in **Fig. 28**. In order to monitor all the sulfate groups in the titration cell, the samples were measured within aqueous media with pH values around 11. Thus, any possible interaction between sulfate and amino groups was avoided (Genco et al. 2012). The total negative charge on the viscose fibers was determined by extrapolating the plateau back to a zero concentration of polyelectrolyte (Wägberg et al. 1989; Laine et al. 1996; Zhang et al. 1994). The negative charge determined for the non-coated fibers B-Vis was around 23.7 mMol kg^{-1} which is almost the same as determined by potentiometric titration. In the cases of the coated fibers BAECS-Vis and AECS-Vis, the negative charge values were higher than in the case of B-Vis. This was expected due to the adsorption of ACS, which

results in the presence of two different types of anionic groups, i.e. the initial carboxyl groups on the fibers weak and the adsorbed strong acids like sulfate groups.

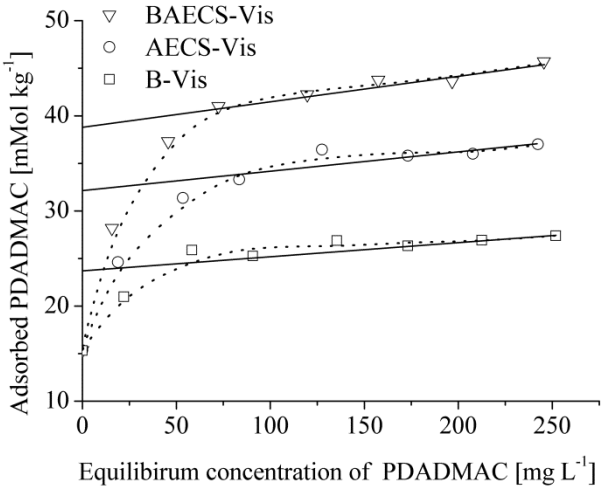

Fig. 28. Adsorption of polydiallyldimethylammonium-chloride (PDADMAC) on non-coated viscose fibers (B-Vis) and coated fibers with 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS-Vis), and with 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS-Vis), as a function of the equilibrium concentration regarding PDADMAC.

This is in agreement with both the titration experiments which showed that the total charge was increased and the XPS results which showed the introduction of sulfate groups. Thus, in the coated fibers, the anionic groups are the sum of the initial fiber carboxyl groups, and the adsorbed sulfate groups. Furthermore, the negative-charge on AECS-Vis 38.8 mMol kg^{-1} was higher than in the case of BAECS-Vis 32.1 mMol kg^{-1}. This was due to the fact that the DS of the sulfate moiety at AECS was higher than in the case of BAECS (**Table 11**).

Table 11. Accessible amounts of sulfate groups determined by polyelectrolyte titration and the methylene-blue method on the non-coated fibers (B-Vis), the coated fibers with 6-deoxy-6-(2-(bis-N´, N´-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS-Vis), and the coated fibers with 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS-Vis).

Sample	Polyelectrolyte titration	Methylene blue method
	Negative charge [mMol kg^{-1}]	Negative charge [mMol kg^{-1}]
B-Vis	23.7 ± 2.7	22.7 ± 1.8
BAECS-Vis	32.1 ± 2.2	29.8 ± 2.0
AECS-Vis	38.8 ± 3.0	34.1 ± 1.4

3.3.6. Methylene-blue method

The negative charges of the viscose fibers were determined by the conventional Methylene blue method (see **Table 11**). For the same reason as mentioned above during the polyelectrolyte titration, the pH values of the aqueous media used in the measures were adjusted to pH 11. The total negative-charges determined on B-Vis, BAECS-Vis, and AECS-Vis were 22.7, 29.8, and 34.1 mMol kg^{-1}, respectively. The larger part of the negative-charge determined on the non-coated fibers B-Vis, originated from carboxyl groups, whereas, in the cases of viscose fibers coated with ACS, the negative charges arose from both the sulfate and

carboxylate groups of the fibers (**Fig. 29**)**.** The results obtained from the spectroscopic methylene-blue method agreed very well with the results obtained from the polyelectrolyte titration (**Table 11**)**.**

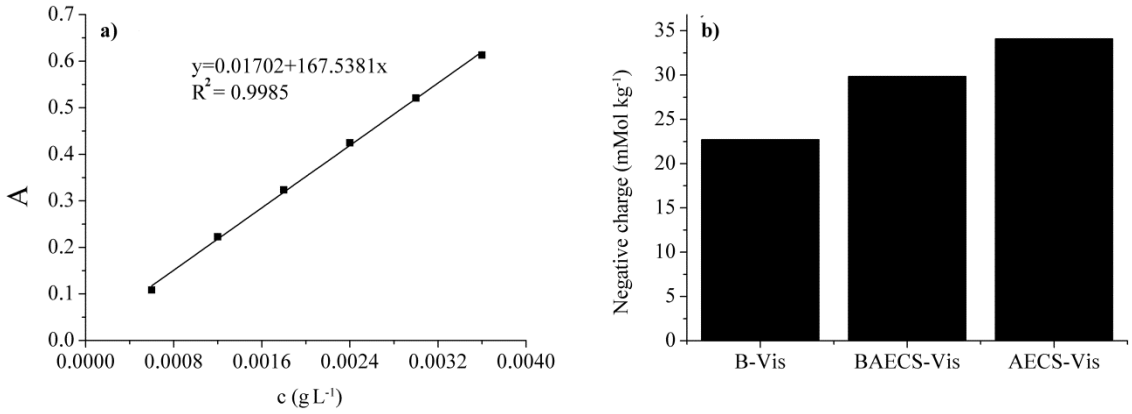

Fig. 29. a) Calibration curve of absorbance against the concentration of methylene-blue within a range between 0.6 and 3.6 mg.L^{-1} b) Negative-charge content in the non-coated fibers (B-Vis), the coated fibers with 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS-Vis), and the coated fibers with 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS-Vis) as determined by the methylene-blue method.

3.3.7. Zeta potential measurements

As shown in **Fig. 30**, the non-coated viscose fibers showed the zeta potential (ζ) behavior as function of the pH value (ζ = f(pH)) typical for viscose cellulose fibers (Stana-Kleinschek et al. 2002; Stana-Kleinschek et al. 2001). ζ for B-Vis was negative within practically the whole pH region, with a plateau value at -10 mV. When the viscose fibers were coated with amino cellulose sulfates, the function ζ(pH) showed typical amphoteric characteristics. The protonation of the amino groups within the acidic region caused the streaming potential to turn positive streaming potential below the isoelectric point, which indicated the amphoteric character of the coated fibers. Both the coated fibers, BAECS-Vis and AECS-Vis, showed a shifting of the isoelectric point into a higher pH region, which is in accordance with the amino group quantities in each sample calculated by conductometric and potentiometric titrations. Negative ζ plateau values were also detected on both the coated viscose fibers' samples, due to the presence of

carboxyl groups and the sulfate groups (see **Fig. 30a**). The isoelectric points obtained from zeta potential measures for B-Vis, AECS-Vis and BAECS-Vis were ~1.7, ~3.1, and ~6.3, respectively. The isoelectric points obtained from zeta potential measures were higher than the PZC's obtained from potentiometric titration, which is probably due to differences in the nature of the techniques (surface potential vs. total charge). Moreover, comparison of the ζ (pH) curves of coated fibers, BAECS-Vis and AECS-Vis, with those of BAECS and AECS solutions, clearly showed that the isoelectric points on the coated fibers were shifted in order to be nearer to the isoelectric point of the ACS in the solution (Genco et al. 2012) (see **Fig. 30b**). It can be concluded from this comparison that BAECS and AECS were adsorbed onto the viscose fibers at a quite high percentage. This comparison gives further qualitative evidence of the existence of amino groups on the coated fibers, and their amphoteric character.

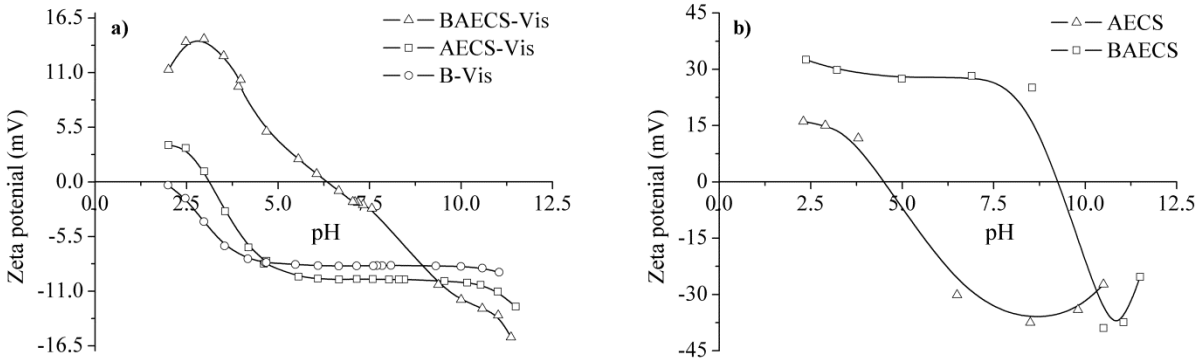

Fig. 30. Zeta potential versus pH value for: (**a**) the non-coated fibers (B-Vis), the coated fibers with 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS-Vis), and the coated fibers with 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS-Vis). (**b**) Aqueous solution of the 6-deoxy-6-(2-(bis-N′,N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS), and the 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS).

3.3.8. Antimicrobial Activity evaluation of AECS-Vis and BAECS-Vis

The antimicrobial activities of ACS-viscose coated fibers were determined by pH=5 and pH=6.8. It is essential to obtain this information when coated-fibers are meant to be used for the development of medical and hygienic textiles that are often applied to the external and internal organs (skin and mucous membranes) that require particular pH values, depending on the physiological pH values of different environments. The antimicrobial activity results for gram-positive bacteria like *Staphylacoccus aureus* and *Streptococcus agalactiae*, for gram- negative like *Eschericha coli*, and for fungi like *Candida glabrata* and *Candida albicans*, was increased using typical behavior when the pH value of the media was changed from 6.8 to 5. This behavior can be ascribed to the fact that, when decreasing the pH values of the mediums of ACS derivatives, the amounts of the protonated amino groups increase. Since the protonated amino groups are responsible for the antimicrobial act (Zhao et al. 2010; Muzzarelli et al. 1986; Shahidi et al. 1999; Goy et al. 2009), the antimicrobial activity increased with decreasing the pH values of the functionalized fiber suspension. Most of the reduction values exceeded 80% at pH 5, which means that the functionalized fiber can be used within the medical industry at pH 5. The only expectation is AECS-Vis (pH=5) where the reduction value for *Candida albicans* was around 50%, which makes inapplicable for medical usage (**Fig. 31**)**.** It is clear that fibers coated by ACS may be used for medical applications; especially for those applications requiring an acidic environment is required.

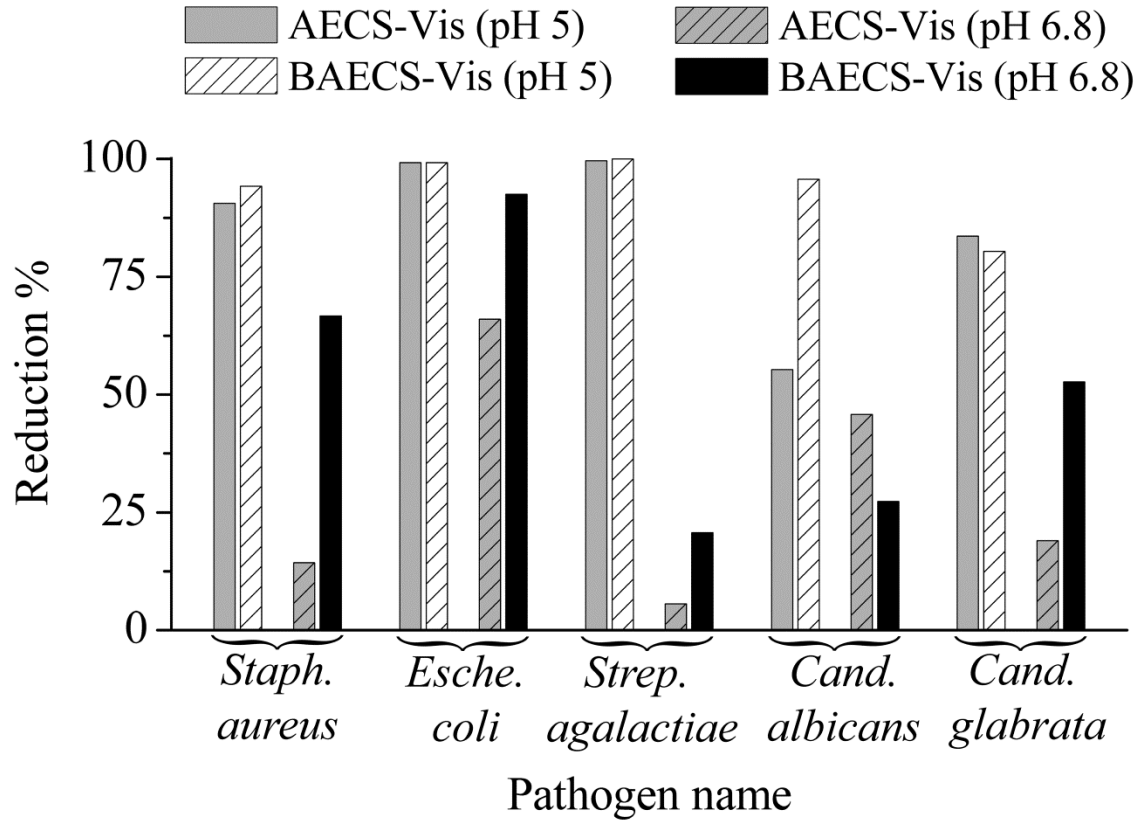

Fig. 31. Antimicrobial impacts of those viscous fibers coated with 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate (BAECS-Vis), and viscous fibers coated with 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS-Vis), on gram-positive bacteria (Staphylacoccus aureus and Streptococcus agalactiae), for gram- negative bacteria (Eschericha coli), and for fungi (Candida glabrata and Candida albicans), measured at pH 5 and 7. The results are expressed as R = % reduction of the organism after contact with the test specimen, compared to the number of bacterial cells surviving after contact with the control.

4. Experimental part

4.1. Synthesis and characterization of aminocellulose sulfates

4.1.1. Materials

Microcrystalline cellulose Avicel® PH 101 was purchased from Fluka (Neu-Ulm, Germany). Determination of the intrinsic viscosity according to ISO 5351 and size exclusion chromatography of a carbanilated sample according to (Terbojevich et al. 1995) yield a degree of polymerization DP_w (ISO) of 264, DP_n (SEC) of 78 and DP_w (SEC) of 281. Cellulose and LiCl (Merck, Darmstadt, Germany) were dried for 6 h at 105 °C under vacuum over potassium hydroxide prior to use. Methyl-6-O-tosyl-a-D-glucopyranoside was purchased from Wako Chemical, Ltd. Other chemicals were purchased from Sigma Aldrich (Deisenhofen, Germany) and were used without further treatment.

4.1.2. Measurements

NMR spectra were acquired in D_2O or dimethyl sulfoxide-d_6 (DMSO-d_6) on a Bruker Avance 250MHz or Avance 400MHz spectrometer with up to 75 000 scans, applying 100 mg sample per mL. FT-IR spectra were recorded on a Nicolet AVATAR 370 DTGS spectrometer with the KBr technique. Elemental analysis was carried out using a Vario ELIII (Elementaranalysensysteme, Hanau, Germany). UV-Vis measurements were performed with a PerkinElmer λ10 UV-Vis spectrometer using quartz glass cuvettes. Size exclusion chromatography (SEC) was carried out in aqueous Na_2HPO_4/NaN_3 buffer system (0.01 M) at pH 11 on a JASCO SEC system with a SEC-pump PU-980, RI detector (RI-930), and UV-detector (UV-975); columns: PSS SUPREMA pre/1000/100 Å; flow rate: 1 mL/min; 30 °C. For the calibration, pullulan (200–700 000 g mol^{-1}) was used as a polymeric standard.

4.1.3. Methods

The degree of substitution of tosyl cellulose (DS_{Tos}) was calculated from the sulfur content according to:

$$DS_{Tos} = \frac{M_{AGU}.w_S(\%)}{M_S.100\% - M_{Tos}.w_S(\%)} \quad (1)$$

M_{AGU} denotes the molar mass of the anhydroglucose unit (AGU), M_S the molar mass of sulfur, M_{Tos} the molar mass of the tosyl group, and $w_S(\%)$ the mass fraction of sulfur in the samples determined by elemental analysis.

The calculation of DS_{Tos} and DS_{Sulf} in tosyl cellulose sulfates was carried out on the basis of UV-Vis measurements and elemental analysis. Therefore, the UV-Vis spectra of ten standard solutions of Methyl-6-O-tosyl-α-D-glucopyranoside (MTG) in deionized water, in a concentration range from 0.010 and 0.045 g/L were measured (**Fig. 32a**). By plotting the absorbance at 227 nm over the concentration range, a calibration curve was obtained (**Fig. 32b**). Utilizing this calibration curve, the concentration of tosyl moieties c_{Tos} can be calculated from the absorbance A_{227} of the solutions of the tosyl cellulose sulfate samples in water:

$$c_{Tos} = \frac{A_{227}+0.0086}{12451} \quad (2)$$

From c_{Tos}, the mass concentration of the tosyl cellulose sulfate sample (c_W) and the molar mass of sulfur M_S, the mass fraction of sulfur in the sample arising from tosyl groups can be calculated:

$$w_S(\%)_{Tos} = \frac{c_{Tos}.M_S.100\%}{c_W} \quad (3)$$

Elemental analysis yields the overall sulfur content $w_S(\%)$ arising from both tosyl and sulfate moieties of the tosyl cellulose sulfate. The difference between the overall sulfur content $w_S(\%)$ and $w_S(\%)_{Tos}$ yields the sulfur content arising from the sulfate groups:

$$w_S(\%)_{Sulf} = w_S(\%) - w_S(\%)_{Tos} \quad (4)$$

and subsequently the concentration of sulfate groups:

$$c_{Sulf} = \frac{w_S(\%)_{Sulf} \cdot c_W}{M_S \cdot 100\%} \quad (5)$$

The concentration of modified anhydroglucose units (AGU), c_{AGU} can be calculated from:

$$c_{AGU} = \frac{c_W - c_{Tos}(M_{Tos} - M_H) - c_{Sulf}(M_{Sulf} - M_H)}{M_{AGU}} \quad (6)$$

Combining the results of eq. (2), eq. (5), and eq. (6) led to the degree of substitution of tosyl cellulose sulfates:

$$DS_{Tos} = \frac{c_{Tos}}{c_{AGU}} \text{ and } DS_{Sulf} = \frac{c_{Sulf}}{c_{AGU}} \quad (7)$$

The sulfur and nitrogen content determined by elemental analysis was used for the calculation of the DS-values of the amino cellulose sulfates (DS_{BAEA}, 6-deoxy-6-(2-(bis-N´,N´-(2-aminoethyl)aminoethyl)) amino cellulose-2,3(6)-O-sulfate and DS_{AEA}, 6-deoxy-6-(w-aminoethyl) amino cellulose-2,3(6)-O-sulfate).

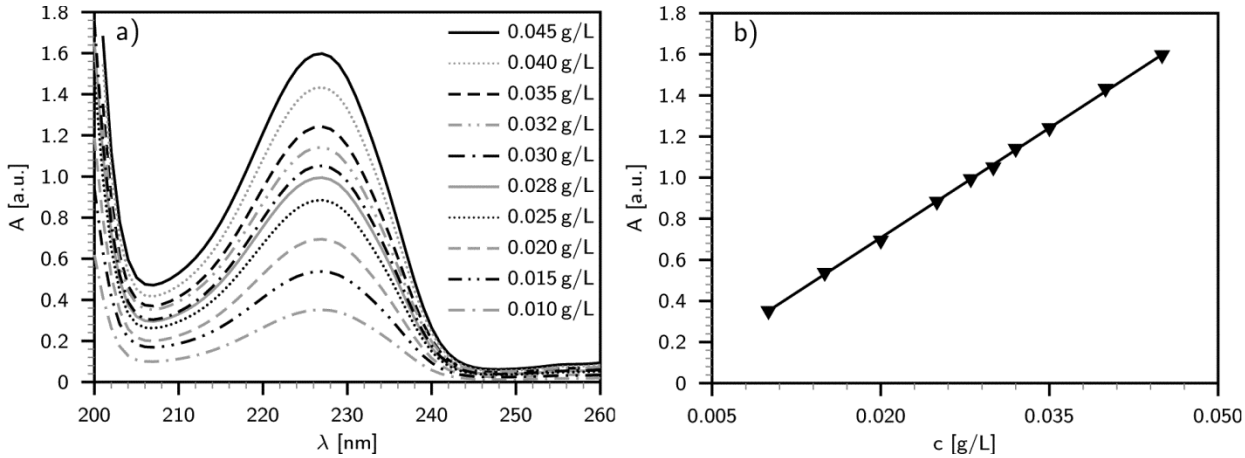

Fig. 32. a) UV spectra of the methyl 6-O-tosyl-a-Dglucopyranoside (MTG) dissolved in deionized water at different concentrations. b) Calibration curve calculated from the absorbance at wavelength 227 nm as a function of the concentration of MTG.

4.1.4. Synthesis

4.1.4.1. Tosyl cellulose **2a-d** and tosyl cellulose sulfates **3a-d**:

Tosyl cellulose (**2**) was prepared under homogeneous reaction conditions according to (Rahn et al. 1996). Briefly, cellulose (**1**) was allowed to react with *p*-toluenesulfonyl chloride and triethylamine as a base for 24 h at 8 °C in N,N-dimethylacetamide (DMAc)/LiCl, (4.3% cellulose content). Tosyl cellulose sulfates (TCS, **3**) were prepared under homogeneous reaction conditions as well. Therefore, **2** was allowed to react with sulfur trioxide pyridine complex using DMAc as the solvent (Heinze and Rahn 1996).

4.1.4.2. 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate **4a-d**, typical example:

TCS 3a (15 g, 43.75 mMol) was dissolved in 105 mL H_2O to give a clear pale yellow solution. The solution was heated at reflux under an inert atmosphere and 1,2-diaminoethane (DAE, 60 mL, 893 mMol) was added drop-wise within 25 min. After 6 h the reaction mixture became a clear pale brown solution. The reaction mixture was cooled to room temperature and the product was precipitated in 1.5 L acetone and washed 4 times with acetone (each 300 mL). Subsequently, the final product **4a** was dried at 50°C under vacuum. Yield: 12 g, 90.6%; EA [%]: C 26.39, H 4.05, N 3.79, S 12.92; DS_{AEA} 0.41, DS_{Tos} 0, DS_{Sulf} 1.25; FT-IR (KBr) $\tilde{\nu}$[cm^{-1}]: 3440 ν(-OH), 3240 ν(NH_2), 2947 ν(CH_2), 1468 ν(NH_2), 1237 ν(SO_2, sulfate), 1142- 1109 ν(C-N primary and secondary), 800 ν(S-O, sulfate). ^{13}C-NMR (D_2O) [ppm]: δ = 100.82 (C1), 83.37–72.50 (C2-C5), 66.59 (C6-OSO_3), 48.73 (C6-N), 45.48 and 38.35 (C7, C8).

4.1.4.3. 6-deoxy-6-(2-(bis-N′,N′-(2-aminoethyl)aminoethyl)) amino cellulose-2,3(6)-O-sulfate **5a-d**, typical example:

To a solution of **TCS 3a** (15 g, 43.75 mMol) in 105 mL H_2O 148 mL tris-(2-aminoethyl)amine (TAEA, 989 mMol) were added dropwise. The reaction mixture was kept at 100 °C for 6 h under a nitrogen atmosphere. After cooling to room temperature, the product was precipitated in acetone (1.5 L) and washed 4 times with acetone (each 300 mL). After filtration, the final product **5a** was dried at 50 °C under vacuum. Yield: 11.8 g, 86%, EA [%]: C 27.99, H 4.28, N 5.75, S 11.46, DS_{BAEA} 0.32, DS_{Tos} 0, DS_{Sulf} 1.21, FT-IR (KBr) $\tilde{\nu}$ [cm^{-1}]: 3481 ν(-OH), 3148 ν(NH_2), 2960 ν(CH_2), 1490 ν(NH_2), 1253 ν(SO_2, sulfate), 1141-1066 ν(C-N primary, secondary and tertiary), 800 n(S-O, sulfate), ^{13}C-NMR (D_2O) [ppm]: δ = 100.40 (C1), 81.73-72.40 (C2-C5), 66.45 (C6-OSO_3), 53.75- 48.92 (C8, C9), 45.46 (C6-N), 38.88-37.32 (C7, C10), 21.14 (C11′).

4.2. Physicochemical properties and bioactivity of aminocellulose sulfates

4.2.1. Materials

6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS), **4c**, DS_{Sulf} = 1.41, DS_{AEA} = 0.85, DS_{Tos} = 0.09] and 6-deoxy-6-(2-(bis-N′,N′-(2-aminoethyl)aminoethyl)) amino cellulose-2,3(6)-O-sulfate [BAECS, **5c**, degree of substitution $(DS)_{Sulf}$ = 1.31, DS_{BAEA} = 0.71, DS_{Tos} = 0.05] were used. Cellulose sulfate (CS, DS_{sulf} 1.74) was synthesized according to the literature (Gericke et al. 2009b). Heparin sodium salt from porcine intestinal mucosa was purchased from Fluka (product 51551). Other chemicals were purchased from Sigma Aldrich (Deisenhofen, Germany) and were used without further treatment.

4.2.2. Polyelectrolyte titration

Polyelectrolyte titrations were carried out in an aqueous media within a pH interval of 4 –11.7. A bulk solution 0.1% (w/v) of the sample with a pH ≈ 11.7 was prepared. Three microliters of this bulk solution was pipetted into the titration cell. Two hundred microliters of 0.1×10^{-3} M

indicator toluidine Blue (T-Blue) were added. The cell was filled to a volume of 40 mL with deionized water and adjusted to the desired pH value with NaOH/HCl (1 M). A Mettler Toledo DL 53 titrator with a 10 mL burette was used for the incremental additions of the polyelectrolyte titrant polydiallyldimethylammonium chloride (PDADMAC) with $c = 1 \times 10^{-3}$ M. Incremental additions of 100 µ L were added every 3–10 s. The absorbance was measured as a potential change in mV, using a Mettler Toledo Phototrode DP660 at a wavelength of 660 nm. The concentration of the accessible sulfate groups was determined from the equivalent volume of the added PDADMAC solution. The negative charge equivalence of the ACS derivatives was calculated from the amount of PDADAMAC needed to reach the point of steepest slope, assuming complete 1:1 ion pair interaction within the polyelectrolyte complex formation. Because the determination of the sulfate group is performed when the products are completely soluble at alkaline pH values, the assumption (1:1 ion pair interaction) is valid as long as the adsorbed polyelectrolyte is bound to the soluble polymers in a flat confirmation.(Horvath et al. 2006; Notley 2008; Wågberg et al. 1987; Winter et al. 1986)

4.2.3. Potentiometric titration

A T70 Titrator with a two-burette instrument (Mettler Toledo T70) was used for potentiometric titrations. The titrator was equipped with a combined glass electrode (Mettler Toledo DG 117). The burettes were filled with 0.1 M HCl and 0.1 M NaOH. The solutions were prepared with deionized and degassed water with low carbonate content ($\approx 10^{-4}$ M). The solution was boiled and subsequently cooled under a nitrogen atmosphere. Twenty milligrams of sample was weighed and diluted with deionized degassed water to a volume of 40 mL in the titration cell. About 0.126 mL of degassed 1 M NaOH was pipetted into a titration cell to give a solution pH of ≈ 11.5. The sample solution was titrated in several forth and back runs between pH 2.5 and 11.5, using HCl–NaOH, at room temperature. The electrode readings were recorded either after a drift

of less than 0.1 mV min^{-1} or the maximum waiting time condition was satisfied. For achieving the equilibrium reading, all the sample solutions were left for about 10 min before starting measurements using stirring and nitrogen purging. Blank titrations were performed under the same conditions as for the samples.

4.2.4. Conductometric titration

The conductometric titrations for the ACS solutions were performed in an aqueous media at pH range (2.7–11.5). Twenty milligrams of the sample was weighed in the titration cell and filled up with deionized and degassed water to a volume of 40 mL to give pH ≈ 11.5. (adjusted by 0.126 mL of degassed 1 M NaOH). The solutions were prepared with deionized and degassed water with low carbonate content (≈ 10^{-4} M); the solution was boiled and subsequently cooled under nitrogen atmosphere. A Mettler Toledo T70 Titrator with a 10 mL burette was used for the incremental additions of hydrochloric acid as a titrant (HCl, c = 0.1 × 10^{-3} M). Incremental additions of 25 μ L were added every 3–10 s. The conductivity was measured using a sensor type Inlab 730, NTC, 0.01–1000 mS cm^{-1}. A blank solution, (0.126 mL of degassed 1 M NaOH was diluted with deionized and degassed water to a volume of 40 mL with pH ≈ 11.5), and titrated in the same way as the ACS solutions.

4.2.5. Zeta Potential Measurements

Electrokinetic measurements have been used in order to define ZP of the products using preparation of 0.1% (w/v) ACS samples in aqueous medium of pH 11.5. ZP (using the Smoluchowski equation)(Sze et al. 2003) was determined within a pH range from 11.5 to 2.3, the pH values were adjusted by adding drops of 1 M NaOH/HCl solution. At each pH value, the measurements were repeated five times using PALS Zeta Potential Analyzer equipped with a

particle sizer from Brookhaven Instruments Corp. An average value of ZP was calculated. The conductivities of the measured solutions were within the range (1–2) mS cm^{-1}.

4.2.6. Antimicrobial activity

Antimicrobial activity was evaluated by modified ASTM E2149-01 under dynamic contact conditions. Gram-positive and Gram negative bacteria, as well as a fungi, were used as test organisms. An incubated test culture in a nutrient broth was diluted using a sterilized 0.3×10^{-3} M phosphate buffer (KH_2PO_4; pH 6.8 and pH 5), in order to provide a final concentration of 1.5–3.0×10^{5} colony forming units (CFU) mL^{-1}. This solution was used as a working bacterial dilution. One microliter of ACS solution 0.5% sample was transferred to a 250 mL Erlenmeyer flask containing 50 mL of the working bacterial dilution. All flasks were loosely capped, placed in the incubator, and shaken for 1h 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 the nutrient agar. The inoculated plates were incubated at 37 °C for 24h and the surviving cells counted. The average values of the duplicates were converted into CFU mL^{-1} in the flasks, by multiplying with the dilution factor. The antimicrobial activity was expressed as R, that is, percent reduction of the organism after contact with the test specimen, compared with the number of bacterial cells surviving after contact with the control (E2149-01 2002).

4.2.7. Antithrombogenic activity

Antithrombogenic activity was detected by measuring the APTT. A Thrombotrack Solo (Axis-Shield PoC AS, Norway) coagulometer was used. Eighty microliters of citrated normal plasma (ORKE 41, Dade Behring), 20 µL of ACS solution and 100 µL Pathromtin SL reagent (OQGS29, Dade Behring) were mixed. After 3 min of incubation at 37 °C, 100 µL of prewarmed

0.025 M $CaCl_2$ (ORHO37 Dade Behring) solution was added to the mixtures to start the coagulation. The end point, which gives the APTT time, was determined automatically by the coagulometer. The final concentration of ACS was adjusted to cover a range from 0 to 200 μg mL^{-1} by dissolution in 0.9% of NaCl aqueous solution at pH 8. Heparin sodium salt was used as a reference, and a blank solution which contained the same components except for ACS, were tested in the same way as the sample solutions.

4.3. Characterization of viscose fibers modified with aminocellulose sulfates

4.3.1. Materials

Amino cellulose sulfate derivatives namely 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate [BAECS **5c**, DS_{Sulf} = 1.31, DS_{BAEA} = 0.71, DS_{Tos} = 0.05] and 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate [AECS **4c**, DS_{Sulf} =1.41, DS_{AEA} = 0.85, DS_{Tos} = 0.09] were synthesized as described by (Heinze et al. 2012). Standard viscose fibers (39 mm, 1.3 dtex) were supplied by Lenzing (Lenzing AG, Austria). A low foaming nonionic scouring agent, Imerol PCJ liq, was obtained from Clariant Textile Chemicals. Methylene blue dye with a grade certified by the Biological Stain Commission, a technical grade poly(vinylsulfonic acid, sodium salt) solution 25 wt. % in H_2O, poly(diallyldimethylammonium chloride) solution with average Mw 200,000-350,000 20 wt. % in H_2O, sodium carbonate BioXtra ≥ 99.0% and all the other chemicals such as sodium hydroxide, potassium hydroxide sodium chloride, potassium chloride, boric acid, and potassium dihydrogen phosphate were purchased as analytical grade reagents from Sigma-Aldrich). Deionized water with low carbonate content (< 10^{-5} M) obtained by boiling the deionized water and cooling under nitrogen atmosphere was used during experiments.

4.3.2. Pretreatment and coating of viscose fibers

Before coating with amino cellulose sulfate, the viscose fibers were pre-treated with alkali (Zemljič et al. 2008). For this, 1.5 g of Na_2CO_3 and 1.5 g of the scouring agent were added to 1.5 L of deionized water, to give a solution with pH 10.9. 50 g of viscose fibers were suspended in the prepared solution. The pretreatment was carried out by inserting the fiber suspension into sealed stainless-steel dye pots housed in a Mathis Labomat BFA dyeing machine at 60 °C for 30 min. After that, the cured fibers were rinsed intensively with deionized water until the conductivity of the suspension was less than 3 μS m^{-1}. Then the fibers were air-dried overnight. 2.5 g of amino cellulose sulfate was dissolved in 250 ml of aqueous NaOH solution at pH 11 in order to obtain a concentration of 10 g L^{-1}. 5 g of the pretreated cellulose fiber were dispersed in the 250 ml of amino cellulose sulfate solution. After shaking for 2h at room temperature, fiber impregnation was performed by passing the suspension through a foulard impregnation press (Werner Mathis) at a pressure of 1.6 bars (~100% wet pick-up). The fibers then were pre-dried at 40 °C for 30 min, and then air-dried overnight.

The ACS-treated fibers were immersed in 250 ml of 0.01 M HCl and stirred for 30 min. The nitrogen content, desorbed from the fibers during soaking in HCl, was detected using a total nitrogen analyzer (TN) from Analytik Jena Multi C/N 2100 Instruments. Thus, the retention value of the adsorbed amounts of ACS on the fiber after washing in HCl was calculated indirectly from the nitrogen content desorbed from the fiber into the washing solution. After that, the coated fibers were rinsed thoroughly with deionized water until the conductivity of the suspension was less than 3 μS m^{-1} and air-dried overnight at room temperature. The moisture content, ~10 % w/w of the studied viscose fibers, was determined using a Mettler Toledo HB43 moisture analyzer, and taken into account during calculations. The samples of 6-deoxy-6-(2-(bis-N′, N′-(2-aminoethyl)aminoethyl))amino cellulose-2,3(6)-O-sulfate coated viscose fibers (BAECS-Vis), 6-deoxy-6-(ω-aminoethyl)amino cellulose-2,3(6)-O-sulfate coated viscose fibers (AECS-Vis), and the non-coated viscose fiber (B-Vis), were then collected for studies.

4.3.3. Characterization of fibers

4.3.3.1. XPS

A XPS (X-ray photoelectron spectroscopy) spectrometer PHI model TFA XPS from Physical Electronics was used to analyze the surfaces of the fibers. The depth of analysis (escape depth of the photoelectrons) in cellulose is a few nm (Buchert et al. 2001; Fras et al. 2005; Johansson et al. 2004; Johansson 2002). The diameter of the analyzed area was about 0.4 mm^2. A monochromatic Al K-Alpha X-ray source (1486.7 eV) was used for excitation. The photoelectrons were detected using a hemispherical analyzer positioned at an angle of 45° with respect to the normal sample surface. The survey scan spectra were obtained within an energy-range of 0-1100 eV, 0.4 eV step, and pass electron energy 187.85 eV, 3 cycles. Spectra were recorded in two different places on the surface and the average atomic composition calculated. In order to obtain the atomic percentage values, each raw XPS signal was corrected by dividing its signal intensity (number of electrons detected) by a relative sensitivity factor (RSF) and normalized over all the elements detected (Ghosh 2009). For determining chemical bonding, high-resolution carbon spectra were recorded within the energy-range 278-296 eV, 0.1 eV step, and pass electron energy 23.5 eV. An average of the spectra determined on three different areas of the fibers was used for analysis. A linear combination of Gaussian and Lorentzian line shapes was used to fit the sub-peaks corresponding to different carbon environments. The full width at half-maximum intensity (FWHM) was about 1.2 eV. A program from the manufacturer Multipack was used for data processing.

4.3.3.2. Potentiometric titration

2 g of the dry viscose fibers were suspended in 200 ml of acidic aqueous solution pH ~ 2.8, adjusted by adding 3 ml of 0.1M HCl. The titration was carried out on a Mettler Toledo T70 titrator equipped with a 10 mL burette filled with 0.1 M KOH. The suspension was titrated from

an initial pH value of 2.8 to pH = 10.5. The titration experiments were carried out at 0.01 M ionic strength, set to its appropriate value with KCl. The titrant was added dynamically within a preset interval of [0.001 – 0.25] mL. The equilibrium criteria for the timed addition was set at d*E*/d*t* = 0.1 mV/150 s. 150s was the minimum time allowed for reaching equilibrium conditions between the two additions of the titrant, and the maximum time was set at 7200 s. The pH value was measured with a Mettler Toledo DG-117 combined glass electrode. Blank titration without the fibers was performed in the same manner, in order to minimize any errors due to the presence of impurities and carbonate ions in the suspension. All the presented values are the mean values of 3 parallel measurements.

4.3.3.3. Conductometric titration

2 g of dry fiber were suspended in 200 ml of aqueous solution at pH ~ 2.5, adjusted by adding 3 ml of 0.1M HCl and ionic strength 0.01 M adjusted with KCl. The suspension was stirred for 30 min, and then titrated with 0.1 M KOH in inert atmosphere (N_2 bubbling). The titrant was added in increments of 25 µL every 3 - 10 seconds. The conductivity was measured using an Inlab 730, NTC, 0.01- 1000 $mS.cm^{-1}$ sensor-type. A stability criterion of 0.1 µS/150 s was set. The amounts of weak acids, like protonated amino groups and carboxyl groups, were obtained by extrapolating the linear parts of the titration curves to their points of intersection (Fras et al. 2004). A blank titration was performed in order to calibrate the system and to eliminate the effects of impurities. The results obtained are the mean values of 3 separate titrations.

4.3.3.4. Polyelectrolyte titration

Polyelectrolyte titration was carried out using a particle charge-detector (Mütek PCD 03 pH) for detection of the zero potential of the fibers, combined with an automatic titration unit (Mettler Toledo, DL 28). 0.3g of the dry fiber was transferred into a 100 ml beaker. A set of seven

beakers was prepared for every sample. 40 mL of 0.1 M NaCl with pH 11 were added to each beaker. The pH was adjusted by means of 0.1 M NaOH. Seven different volumes between (0.5 - 10.5) mL of the cationic polymer polydiallyldimethylammonium-chloride (PDADMAC) 1.4144 g. L^{-1} were added to each set. The suspensions were stirred for 30 min in order to reach adsorption equilibrium and then filtered through filter paper using a Büchner funnel. The fibers were then washed with some more water so that the total amount of filtrate was 55 mL. 10 mL of filtrate (which contains a non-adsorbed polyelectrolyte) were titrated with poly(vinylsulfonic acid, sodium salt). A set of seven blank samples, which contained the same volumes of cationic polymer without fiber, were titrated in the same way as the fibers so that the effects of polymer adsorption by the glassware and the glass-fiber filter could be eliminated. The filtrated samples were dried in an oven at 105°C for at least 4 h. The dried samples were placed in a dry desiccator to cool down, and were then weighed. The following equation was used for calculating the charge (Q) of the fibers:

$$Q = \frac{(V_0 - V_{tit}).c_{pvs}}{m_{dry}} . \frac{V_{tot}}{V_{sample}} \quad (8)$$

where V_0 is the volume of PVSNa consumed by the blank sample, V_{tit} is the volume of the PVSNa solution consumed during titration of the fiber sample, c_{pvs} is the concentration of the titrant PVSNa, V_{tot} is the total volume of filtrate, V_{sample} is the volume of the filtrate suspension taken to the titration, and m_{dry} is the dry mass of the sample. The charge on the fibers was estimated from the adsorption isotherms by extrapolating the plateau level of adsorption to the zero polymer concentration. The amount of charge adsorbed with the polymer at this point in mMol kg^{-1}, was taken as the charge of the fibers. All reported values are the mean values of three parallel measurements. For a detailed description of the techniques see (Laine and Stenius 1997).

4.3.3.5. Spectroscopic methylene blue method (adapted from Klemm et al. 1998a)

The negative charge of the viscose fibers was determined using a modified conventional methylene-blue method. 0.5g of the fiber were suspended in 25 ml of aqueous methylene blue chloride solution (300 $mg.L^{-1}$) and 25 ml of the prepared buffer of pH=11 for l h at 20°C in 100 ml Erlenmeyer flask. The viscose fiber sample was then isolated via filtration on a sintered-glass disk. 5 ml of the filtrate was transferred to a 100 ml volumetric flask. Then 10 ml of 0.1M HCl and, subsequently, deionized water was added to achieve a total volume of 100 ml. The methylene-blue content of the liquid was determined photometrically at 660 nm, the same selected wavelength as the standards. The total amount of the non-sorbed methylene-blue was calculated using a calibration curve. Thus, the total negative charges' (as a sum of the carboxyl and sulfate groups) content of the viscous fibers was obtained.

4.3.3.6. Zeta potential measurements (adapted from Reischl et al. 2006)

0.5 g of the fiber samples, B-Vis, BAECS-Vis or AECS-Vis, were soaked in 10^{-3} M KCl solution for 60 min prior to the measurements, which enabled full swelling of the fibers (Stana-Kleinschek et al. 2001). Thus, no additional structural changes due to swelling occurred during the actual determination of their zeta potential. The fibers were then placed in the cylindrical cell of the streaming potential measuring device ZetaOszi designed according to (Reischl et al. 2008). Both the upper and lower sieves of the cylindrical cell were adjusted in order to obtain a compressed fiber plug of about 0.5 cm thick. A tubular reservoir was connected to the fiber cells and 100 ml of electrolyte solution (10^{-3} M KCl) was poured into the reservoir. A MultiSens electrode was inserted into the electrolyte solution. The electrolyte solution was then oscillated back and forth through the cell at a frequency of 0.4 Hz for 120 steps. Measuring was started when the air-bubbles trapped inside the fiber sample were fully removed by this oscillation. The starting pH value of the electrolyte solution was adjusted manually to about pH ~ 11.5, by

addition of 1M NaOH solution. Three subsequent single measurements of the zeta potential were made during a period of 60 s, after which a certain amount of 0.1 M HCl solution was added to lower the pH value. The mean value from three measurements was calculated for each additional step. Three repeats were carried out for each sample, resulting in three ZP/pH curves. As a result, the mean curve of ZP=f (pH) is given.

4.3.3.7. Antimicrobial Activity

The antimicrobial properties of the functionalized fibers were evaluated by (ASTME2149-01 2001), which is a quantitative antimicrobial standard test method performed under dynamic contact conditions. Gram-positive bacteria like *Staphylococcus aureus* (*Staph. Aureus*) *and Streptococcus agalactiae* (*Strep. agalactiae*), gram-negative ba

cteria like *Escherichia coli* (*Esche. coli*) and fungi like *Candida albicans* (*Cand. albicans*) and *Candida glabrata* (*Cand. glabrata*), were used as test organisms. An incubated test-culture within a nutrient broth was diluted using a sterilized 0.3 mMol phosphate buffer (KH_2PO_4) at pH = 6.8, in order to give a final concentration of 1.5-3.0 x 105 colony forming units (CFU).mL^{-1}. This solution was used as a working bacterial dilution. Each sample (0.5 g) was cut into small pieces (1 × 1 cm) and transferred to a 250 mL Erlenmeyer flask containing 50 mL of the working bacterial dilution. All the 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. 1 mL of the diluted solution was plated in nutrient agar after a series of dilutions using the buffer solutions. The inoculated plates were incubated at 37°C for 24 h and the surviving cells counted. The average values for the duplicates were converted into CFU.mL^{-1} in the flasks, by multiplication using the dilution factor. The antimicrobial activity was expressed as R = % reduction of the organism after contact with the test specimen, compared to the number of bacterial cells surviving after contact with the

control. The same procedure was applied on an incubated test culture within a nutrient broth diluted in a sterilized 0.3 mM phosphate buffer at pH = 5.

5. Summary

Amino cellulose sulfates, a new class of polyampholytic polysaccharide derivatives, are accessible by a three step synthesis; tosylation, sulfation and nucleophilic displacement for introduction of amino moieties. Thus, tosylate moieties can be introduced in position 6 of the repeating unit of the cellulose backbone. Subsequently, anionic sulfate groups can be linked to the tosyl cellulose by the treatment with sulfur trioxide pyridine complex. The nucleophilic displacement of the tosyl moieties by multifunctional amines like 1,2-diaminoethane or tris(2-aminoethyl) amine leads to amino cellulose sulfate of different structure; namely 6-deoxy-6-(*ω*-aminoethyl)amino cellulose-2,3(6)-O-sulfate (AECS) and 6-deoxy-6-(2-(bis-N′,N′-(2-aminoethyl) aminoethyl)) amino cellulose-2,3(6)-O-sulfate (BAECS) were prepared under homogeneous reaction conditions.

The structure of the polysaccharide derivatives can be clearly described by FT-IR and NMR spectroscopy. Investigation of the solubility of the polymers shows a polyampholyte-typical water solubility that depends on the pH value of the system. This pH value dependency can be tailored by the choice of the amine used for functionalization and the synthesis conditions applied. Moreover, preliminary experiments of the determination of the apparent molecular sizes of the products were carried out applying size exclusion chromatography. The M_W values obtained are 70,490 and 167,700 g mol^{-1} for (AECS **4a**, degree of substitution, DS_{Sulf} = 1.25, DS_{AEA}= 0.41, $DS_{Tos} \approx 0$) and (BAECS **5a**, DS_{Sulf} = 1.21, DS_{BAEA} = 0.32, $DS_{Tos} \approx 0$), respectively. Considering the molar mass of the repeating unit, the corresponding DP_W values are 233 for **4a** and 536 for 5a. In comparison with the DP_W of the initial cellulose (≈ 260–280), a slight decrease can be noticed in case of **4a** and an increase became apparent in case of **5a**. However, the trend of an increasing molecular weight observed for BAECS might be attributed to aggregation resulting from the polyampholytic character of the ACS derivatives. The higher extent of aggregation and thus the higher molecular weight in case of BAECS result from the higher

content of protonated amine groups present in the polymer. The physicochemical behavior of 6-deoxy-6-(2-(bis(2-aminoethyl)aminoethyl-amino) cellulose sulfate and 6-deoxy-6-(2-aminoethyl) cellulose sulfate was studied by means of polyelectrolyte-, potentiometric-, and conductometric titrations as well as by zeta potential measurements. It was found that there is aggregation between oppositely charged sulfate and amino moieties dependent on the pH value. The amounts of protonated amino groups determined by conductometric titration were 4.02 and 2.53 mMol g^{-1} for BAECS and AECS, respectively. Since for both samples the amounts of amino groups are higher than the amounts of sulfate groups, protonation of the excess of amino groups at pH values lower than the isoelectric points causes the products solutions to be positively charged. The antimicrobial test of BAECS and AECS solutions showed that the samples inhibit the growth of *Staphylococcus aureus* and *Candida glabrata* at pH =5 but they are inactive against *Escherichia coli*. Due to blocking of most of the sulfate groups by aggregation with amino/ammonium groups, the products showed no antithrombogenicity. It was possible to control the antithrombogenicity of the products by variation of the DS values of both amino and sulfate groups.

Viscose fibers were coated with BAECS and AECS. The determination of accessible amounts of sulfate (anionic) and protonated amino groups (cationic) on the fibers shows that the amino cellulose sulfates had adsorbed on the fibers leading to amphoteric characteristics. The coating of fibers by AECS and BAECS introduces new functional groups to the fibers; as positively-charged amino groups and negatively-charged sulfate groups. The functional groups of the non-coated fibers and of the ACS-coated fibers were characterized by means of X-ray photoelectron spectroscopy (XPS), conductometric, potentiometric and polyelectrolyte titrations, as well as conventionally by the spectroscopic methylene-blue method. The electrokinetic behavior was evaluated by measuring the zeta potential of the fibers as a function of pH between pH 11.5 and 2.0. The amounts of the positive-charges (protonated amino groups) determined by potentiometric titration agreed with the amounts of the positive charges determined by

conductometric titration. The total amounts of negatively-charged fiber groups (sulfate and carboxyl) determined by polyelectrolyte titration were 38.8 and 32.1 mMol Kg^{-1} for viscose fibers coated with AECS and BAECS, respectively. These results were in accordance with the conventional methylene-blue method. Protonation of the amino groups on the fibers render them positive at pH values lower than the isoelectric point. At pH values higher than the isoelectric point, the fibers are negatively-charged due to the existence of deprotonated sulfate- and carboxyl groups. The antimicrobial test of viscose fibers coated with BAECS and AECS showed that the samples inhibited the growth of *Staphylacoccus aureus, Streptococcus agalactiae, Eschericha coli, Candida glabrata* at pH=5, but the viscose fibers coated with BAECS and AECS were inactive against *Candida albicans* at this pH value.

6. Zusammenfassung

Aminocellulosesulfat, eine neue Klasse von polyampholytischen Polysaccharidderivaten sind in einer dreistufigen Synthese zugänglich: Tosylierung, Sulfatierung und nukleophile Substitution zur Einführung der Aminogruppen. Die Tosylgruppen werden an Position 6 der Wiederholungseinheit des Celluloserückgrates gebunden. Nachfolgend werden anionische Sulfatgruppen durch Umsetzung mit Schwefeltrioxid-Pyridin-Komplex eingeführt. Die nukleophile Substitution der Tosylgruppen durch multifunktionelle Amine, wie 1,2-Diaminoethan oder tris(2-Aminoethyl)amin ergeben Aminocellulosesulfate unterschiedlicher Struktur. 6-Deoxy-6-(*ω*-aminoethyl)aminocellulose-2,3(6)-O-sulfat (AECS) und 6-Deoxy-6-(2-(bis-N´,N´-(2-aminoethyl)aminoethyl))aminocellulose-2,3(6)-O-sulfat (BAECS) wurden so unter homogenen Reaktionsbedingungen synthetisiert.

Die Struktur der Polysaccharidderivate kann mittels FT-IR- und NMR-Spektroskopie eindeutig beschrieben werden. Die Untersuchung der Löslichkeit der Polymere zeigt das für Polyampholyte typische pH-Wert-abhängige Verhalten. Diese Eigenschaft kann durch die Wahl des Amins und die Reaktionsbedingungen gesteuert werden. Darüber hinaus wurden orientierende Versuche zur Bestimmung der scheinbaren Molmasse mittels Größenausschlusschromatographie unternommen. Die erhaltenen Werte betragen 70.490 g mol^{-1} (AECS **4a**, Substitutionsgrad, DS_{Sulf} = 1.25, DS_{AEA} = 0.41, $DS_{Tos} \approx 0$) und 167.700 g mol^{-1} (BAECS **5a**, DS_{Sulf} = 1.21, DS_{BAEA} = 0.32, $DS_{Tos} \approx 0$). Unter Berücksichtigung der molaren Masse der Wiederholungseinheit ergeben sich die korrespondierenden DP_w-Werte von 233 (**4a**) und 536 (**5a**). Im Vergleich zum DP_w der Ausgangscellulose (≈ 260–280), kann bei Probe **4a** ein leichter Polymerabbau beobachtet werden, während der scheinbare DP bei Probe **5a** zunimmt. Die steigende Molmasse von BAECS kann auf den polyampholytischen Charakter dieses Derivates zurückgeführt werden. Die stärkere Aggregation und damit die höhere Molmasse resultiert aus dem höheren Gehalt an protonierten Aminogruppen im Polymer. Das

physikochemische Verhalten von 6-Deoxy-6-(2-(bis(2-aminoethyl)aminoethyl-amino)cellulosesulfat und 6-Deoxy-6-(2-aminoethyl)cellulosesulfat wurde mittels Zetapotentialmessung, Polyelektrolyttitration, sowie potentiometrischer und konduktometrischer Titration untersucht. Dabei konnte eine Aggregation zwischen gegenseitig geladenen protonierten Amino- und Sulfatgruppen beobachtet werden, die vom pH-Wert abhängig ist. Die mittels konduktometrischer Titration bestimmten Gehalte an protonierten Aminogruppen betragen 4,02 mMol g^{-1} (BAECS) und 2,53 mMol g^{-1} (AECS). Weil beide Proben einen höheren Gehalt an Aminogruppen als Sulfatgruppen aufweisen, liegt das Produkt bei pH-Werten, die kleiner als der isoelektrische Punkt sind, protoniert vor. Beide Proben hemmen das Wachstum von *Staphylococcus aureus* und *Candida glabrata* bei pH =5, sind aber unwirksam gegenüber *Escherichia coli*. Aufgrund der Blockierung vieler Sulfatgruppen durch Aggregation mit Amino-/Ammoniumgruppen, wirken die Proben nicht antithrombogen. Die antithrombogene Wirkung kann durch Variation der DS-Werte von Amino- und Sulfatgruppen gesteuert werden.

Viskosefasern sind mit BAECS und AECS beschichtet worden. Die Bestimmung der verfügbaren Mengen an Sulfat- (anionisch) und protonierten Aminogruppen (kationisch) auf den Fasern zeigt die Beschichtung der Fasern an und verleiht diesen amphotere Eigenschaften. Hierdurch werden neuartige funktionelle Gruppen eingeführt: positiv geladene Aminogruppen und negativ geladene Sulfatgruppen. Die funktionellen Gruppen der unbeschichteten und ACS-beschichteten Fasern wurden mittels Röntgenphotoelektronenspektroskopie (XPS), durch Titration (konduktometrisch, potentiometrisch, Polyelektrolyt-) sowie spektroskopisch durch die Methylenblau-Methode bestimmt. Das elektrokinetische Verhalten der behandelten Fasern wurde durch Messung des Zetapotentials als Funktion des pH-Wertes zwischen pH 11,5 und 2,0 beurteilt. Die mittels potentiometrischer Titration bestimmten Gehalte an positiven Ladungen (protonierte Aminogruppen) stimmen mit den durch konduktometrische Titration bestimmten Gehalten überein. Die durch Polyelektrolyttitration bestimmte Gesamtmenge der negativen

Ladungen auf den Viskosefasern (Sulfat- und Carboxylgruppen) beträgt 38,8 mMol kg^{-1} (AECS) bzw. 32.1 mMol kg^{-1} (BAECS). Diese Ergebnisse stimmen mit den Resultaten der Methylenblau-Methode überein. Die Fasern werden durch Protonierung der Aminogruppen bei pH-Werten unterhalb des isoelektrischen Punktes positiv. Bei pH-Werten oberhalb des isolelektrischen Punktes werden die Fasern durch deprotonierte Sulfat- und Carboxylgruppen negativ. Untersuchungen zur antimikrobiellen Aktivität zeigten, dass die Proben das Wachstum von *Staphylacoccus aureus, Streptococcus agalactiae, Eschericha coli*, und *Candida glabrata* bei pH=5 hemmen, aber inaktiv gegenüber *Candida albicans* sind.

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9. Curriculum vitae

Personal Information

Date of birth	20. June 1974
Place of birth	Kafartakharim, Idlip, Syria
Nationality	Syrian
Marital status	Single

Educational background

1999- 2003	Studies of B.Sc. in Chemistry and Physics at the Faculty of Science, University of Aleppo (grade "very good")
2003- 2004	Studies of Diplom in Chemistry at the Faculty of Science, the University of Aleppo; topic “Practical and theoretical fundamentals of instrumental analysis” (grade "excellent")
2004- 2007	M.Sc. thesis at the Faculty of Science, the University of Aleppo; topic “Determination of some pharmaceutical components using pulse voltammeteric analysis” (grade "excellent")
2008- 2013	Scientific co-worker and PhD student in the research group of Prof. Thomas Heinze at the Institute of Organic and Macromolecular Chemistry, Friedrich-Schiller University of Jena, Germany

Work experience

2002 (summer)	Training in pharmaceutical Company, T3A industrial, Asyut, Egypt
2005- 2006	Research Assistant in the laboratory of environmental chemistry at the Faculty of Science, University of Aleppo. (1st semester)
2006- 2007	Teacher of chemistry and physics in secondary schools, Aleppo
2007- 2008	Research Assistant in the laboratory of instrumental analysis at the Faculty of Science, University of Aleppo university. (1st semester)
2009- 2013	PhD student and scientific co-worker at the Friedrich Schiller University of Jena and the University of Maribor

Jena, 17.01.2013

(Taha Genco)

10. Publication list

Publications

- Heinze T, Genco T, Petzold-Welcke K, Wondraczek H (2012) Synthesis and characterization of aminocellulose sulfates as novel ampholytic polymers. Cellulose 19 (4):1305-1313.
- Genco T, Zemljič L, Bračič M, Stana-Kleinschek K, Heinze T (2012) Physicochemical Properties and Bioactivity of a Novel Class of Cellulosics: 6-Deoxy-6-amino Cellulose Sulfate. Macromol Chem Phys 213:539-548.
- Genco T, Zemljič L, Bračič M, Stana-Kleinschek K, Heinze T (2012) Characterization of viscose fibers modified with 6-deoxy-6-amino cellulose sulfate. Cellulose 19 (6):2057-2067.
- Ramadan AA, Mandil H, Genco T (2009) Determination of carbinoxamine maleate in pharmaceuticals by direct and differential pulse polarography. Asian J Chem 21 (9):7387-7397.

Posters and oral presentations

- Taha Genco, Lidija Fras Zemljič, Katrin Petzold-Welcke, Karin Stana-Kleinschek , Thomas Heinze "Amino cellulose sulfate: Synthesis- Adsorption on viscose fibers" Oral Presentation in Fourth Mid-year of STEP-ITN meeting, University of Innsbruck, Dornbirn, Austria,13.-14. 03. 2012
- Taha Genco, Lidija Fras Zemljič, Katrin Petzold-Welcke, Karin Stana-Kleinschek , Thomas Heinze "Physico- chemical behavior of novel aminocellulose sulfate: characterization and potential application" Oral and poster Presentations in the Third End-year of STEP-ITN meeting, University of Maribor, Maribor, Slovenia, 20.-23. 09. 2012

- Taha Genco, Lidija Fras Zemljič, Katrin Petzold-Welcke, Karin Stana-Kleinschek , Thomas Heinze "Amino cellulose sulfate; characterization of amino and sulfate groups" Poster presentation in EPNOE 2011, the 2nd EPNOE International Polysaccharide Conference, Wageningen, Netherlands, 29.08 -02. 09. 2011
- Taha Genco, Lidija Fras Zemljič, Katrin Petzold-Welcke, Karin Stana-Kleinschek , Thomas Heinze " Viscose fiber coated with amino cellulose sulfate: physico-chemical characterization and potential applications" Oral Presentation in Third Mid-year of STEP-ITN meeting, Institute of Biopolymers and Chemical Fibers, Łódź, Poland, 15. - 17. 03. 2011
- Taha Genco, Lidija Fras Zemljič, Katrin Petzold-Welcke, Karin Stana-Kleinschek, Thomas Heinze "Determination of amino and sulfate groups in amino cellulose sulfate by different titration methods" Poster presentation in IPTB 2011, the 7th International Conference on Polymer and Textile, Milan, Italy, 02. - 04. 3. 2011
- Taha Genco, Lidija Fras Zemljič, Katrin Petzold-Welcke, Karin Stana-Kleinschek, Thomas Heinze "Physico- chemical behavior of novel aminocellulose sulfate: characterization and potential application" Oral Presentation in the Second End-year of STEP-ITN meeting, MINES ParisTech/CEMEF, Sophia Antipolis, France, 28. 09 -01.10. 2010
- Taha Genco, Katrin Petzold-Welcke, Thomas Heinze "Aminocellulose sulfate as novel ionic polymers" Oral and poster Presentations in the Second Mid- year of STEP-ITN meeting, Unilever Food and Health Research Institute, Vlaardingen, Netherlands, 29. -31. 03. 2010
- Taha Genco, Katrin Petzold-Welcke, Thomas Heinze "Novel Aminocellulose Derivatives" Oral Presentation in the First End- year of STEP-ITN meeting, Friedrich Schiller University of Jena, Germany, 29. 09 -01.10. 2009

- Abdul Aziz Ramadan, Hasna Mandil, Taha Genco “polarographic methods for determination of pharmaceutical” Oral presentation in SCC II 2008, the Second Syrian Chemical Conference , Faculty of Science, University of Aleppo, Syria, 28. -30. 04. 2008

11. Selbstständigkeitserklärung

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

Jena, den 17.01.2013

(Taha Genco)