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Cellulose Fibres Functionalised by Chitosan: Characterization and Application

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1. Introduction

Fibrous (textile) materials, such as used in the medical field or health services can be divided into two basic groups, according to whether they are used 1) inside organic tissues (internal/implantable): vascular grafts, meshes, stents, tendons and ligament implants, surgical threads, etc.) or 2) on their surface (external/non-implantable): gauzes, bandages, surgical covers, nappies, tampons, etc. The use of natural cellulose fibers, such as cotton and flax, goes back in medical applications to ancient times and still today, in some medical applications, cellulose fibrous materials represent materials that can not be exchanged with any other. In the more recent past new procedures and technologies enabled the production of various chemical cellulose fibers such as viscose, modal and lyocell, which are cleaner and even more hygroscopic than cotton, and as such highly applicable within hygiene and medical fields. The great potential of cellulose fibers lies in their molecular structure, which offers excellent possibilities as a matrix for the design of bioactive, biocompatible, and intelligent materials.

Over the last twenty years the increase in the number of microbially caused diseases and hospital infections has led to intensive research into new materials and procedures, which would assure permanent bioactive effects together with complete safety for the people (Chung et al. 1998, Liu et al. 2000, Lee et al. 1999). Fungi, as well as gram positive and gram negative bacteria are commonly found in textile materials, especially in bedclothes. Many of these microorganisms are pathogens or potential pathogens and quite often related to nosocomial infections (Takai et al. 2002). Fibrous materials used in the medical and hygiene fields are usually in contact with extremely contaminated environments. *Escherichia Coli* and *Penicillium Crysoenum* very often cause diseases, as well as the disintegration and foul smell of fibrous materials (Chang et al. 1996). Therefore, the most important biomedical characteristics of a fibrous material for external use are its' antimicrobial properties. In textiles these are usually achieved by treatments with silvers salts, quaternary ammonium chloride, metals, aromatic, halogen compounds, etc. (Kenawy et al. 2007, Takai et al. 2002). Antimicrobial agents act either as growth inhibitors (bacterio- or fungi-static) or they kill the microorganisms (bio-cidal). Almost all antimicrobial agents used in conventional textile treatments are biocidal, as they damage the cell wall or the membrane permeability, denature proteins, inhibit enzyme activity or lipid synthesis, which are all essential for cell survival (Gao et. al. 2008). The most important issue regarding biocides used on commercial textiles is the potential induction of bacterial resistance to these substances, which could also

lead to increased resistance to certain antibiotics in clinical use, especially because of large quantities of these substances, which are needed for conventional textile treatments. Furthermore, some of these substances produce unpleasant odour and/or discoloration of textiles (N-halamine), need to be regenerated in bleaching process (N-halamine, peroxyacids), or can be depleted (silver) (Gao et al. 2008).

All the mentioned factors have resulted in a high interest in researching and developing technologies, which are based on the use of alternative natural materials. One of such natural polymers is chitosan, which has received lots of attention due to its non-toxicity, antimicrobial properties and wide possibilities for chemical derivations (Berger 2004, Uragami 2006, Muzzarelli 2005, Alonso et al. 2009). The antimicrobial activity of chitosan is assigned to its amino groups, which in diluted acids form ammonium salts. The manipulation of chitosan's binding strategies onto cellulose surfaces i.e.: (i) reversible binding - which enables the release of a bioactive substance from a fibre's surface and, (ii) irreversible binding resulting in the permanent bioactivity of a fibrous (textile) surface, determines the applicabilities of such surfaces.

Efficient and permanent fixation of chitosan is of major importance in the cases of conventionally applied (washable) textiles. Many different approaches have been used in order to permanently bind chitosan to cotton fibers (Fras-Zemljic et al. 2004, El-ahlavy 2005, Strnad et al. 2008). Successful permanent chitosan binding with a large number of accessible amino groups requires a certain amount of anchoring sites on the fibrous substrate. However, interactions with the anchoring groups lower the free amino groups' amount and as such decreases the surface antimicrobial activity of the treated fibres. In order to achieve satisfactory results, it is extremely important to balance between treatment resistance, materials' mechanical properties, and the number of free amino groups in/on the treated material.

2. Cellulose fibers in medical applications

The most important cellulose fibres in medical applications are natural cotton fibers and different regenerated (man-made) cellulose fibres such as viscose, modal, and lyocell. These fibres find medical application in two somewhat different fields: 1) *hygiene and healthcare* (bed linen, mattress covers, incontinence care pads, nappies, tampons, etc.) and 2) so-called *external* textile materials (wound dressings, bandages, gauzes, etc.) The most important characteristics, which determine the medical applications of these fibres, are: moisture and liquids' adsorption, antistatic behaviour, low content of dyes and other chemicals, high mechanical stability, and ease of laundering and/or sterilisation (Rajesh 2006).

A cotton fibre is a single cell, which grows on the seed's surface of a plant from the genus *Gossypium*. At the growth beginning the outer primary wall of a cell grows in length for about 16 - 17 days and after that the secondary wall begins to form with the deposition of concentric layers of cellulose lamella from the outside towards the lumen. The secondary wall in a mature fibre represents the body of the fibre. The primary-wall is composed of cellulose, fats, waxes, pectic substances, and proteinaceous matter, while the secondary-wall is considered to be pure cellulose (De Gruy et al. 1973). During growth, the internal central canal (lumen) is large and filled with plant juices from the protoplasm of the cell. When the growing period ends, the fibers dry out and collapse into shrivelled, twisted, and flattened tubes (Fig. 1). Cotton fibres are around 10 - 55 mm long and with a diameter between 10 and 40 μm . The cross-sections of cotton fibres are elliptical or bean-shaped, with the narrow lumen in the middle.

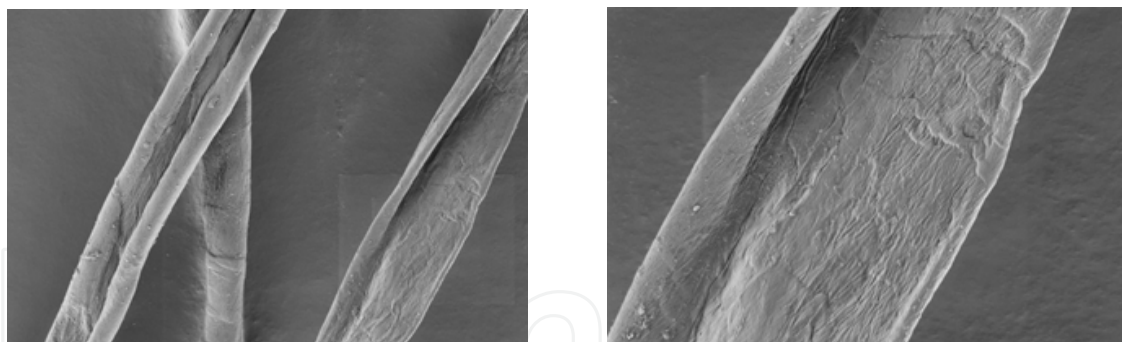


Fig. 1. SEM micrograf of cotton fibre: a) magnification 10 000X and b) magnification 30 000 X

Cellulose macromolecules organise themselves into well-ordered crystalline regions and less ordered amorphous regions. These differently ordering levels alternate along fibrils, which compose fibril bundles and larger fibril structures up to the cell walls. The crystalline unit of natural cellulose (cellulose I) has monoclinic symmetry (Krässig 1993). The main intermolecular connections are H-bonds. During special alkaline treatments, natural cellulose converts into cellulose II, which is also characteristic for regenerated cellulose fibres.

Owing to hemicelluloses, waxes, and other impurities in the primary-wall, raw cotton fibres have rather hydrophobic characteristics. Some pretreatment procedures of fibres are needed in order to purify and to increase the hydrophilicity of cotton fibres. Scouring is an alkaline treatment in NaOH solutions with concentration $< 10\%$, by which impurities and waxes are removed and fibre hydrophilicity is increased. Mercerization, however, is an alkaline treatment at NaOH conc. $> 10\%$, by which the supermolecular structure and fibre morphology are changed and, owing to these changes, the mechanical properties, accessibility to aqueous media, affinity to dyes, and lustre are increased (Šauperl 2009). Besides this, cotton usually needs to be bleached and treated in order to improve its wrinkle resistance.

Another group of cellulose fibres applied in medicine is regenerated (man-made) cellulose fibres (Fig. 2). The main sources of the cellulose for regenerated fibre production are highly refined wood pulps and, to a lesser extent, cotton (Krässig 1993). Conventional regenerated cellulose viscose fibres, are generally produced by the indirect viscose process, based on deriving cellulose from carbon bisulphite, and a modal fibre is produced using a modification of this basic procedure (Cook 1984). Owing to considerable environmental problems connected to viscose process, several decades ago lyocell fibres were produced on the basis of an environmentally-friendly procedure, where the cellulose was dissolved directly in the organic solvent N-methylmorpholine-N-oxide, without the formation of derivatives (Albrecht et al. 1997, Berger 1994, Cole 1994, Krüger 1994). Different production processes regarding viscose, modal and lyocell cause differences in the molecular and supermolecular arrangements of the fibres despite the same chemical composition. The degree of crystallinity of lyocell fibres is approximately 43 % higher than of viscose and about 16 % higher when compared to modal (Kreze & Malej 2003). The same trend can be observed regarding molecular orientation. Fibre sorption properties are, in addition to size and orientation of amorphous regions, influenced by void fracture (diameter, volume, and specific inner surface). Owing to the similar void structures in lyocell and viscose fibres, the sorption, swelling and dyeing properties of these two fibres are similar (Kreze et al. 2001). Lyocell fibres are superior in comparison to viscose in regard mechanical properties. The wet state mechanical properties of fibres are of crucial importance for medical applications.

In the wet-state, lyocell fibres keep over 90 % of their tensile strength in a conditioned state, viscose about 60 %, and modal about 50 % (Kreže & Malej 2003).

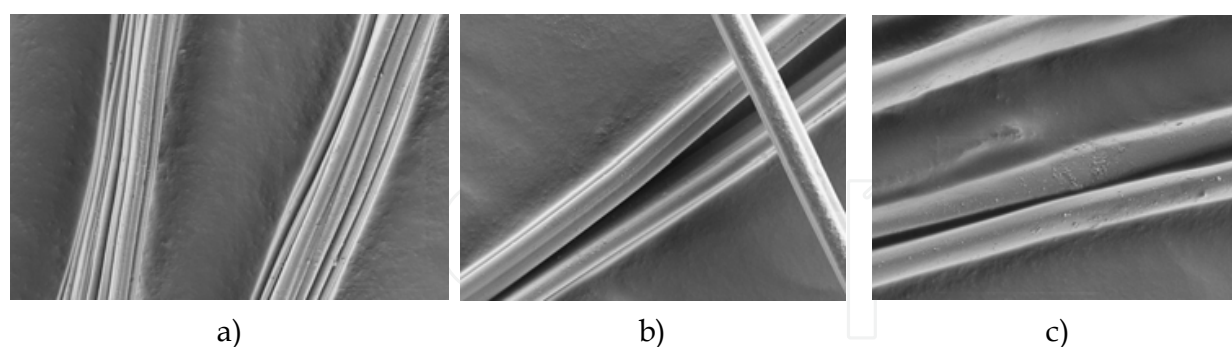


Fig. 2. SEM micrographs of a) viscose, b) modal and c) lyocell fibre at magnification 10 000X

3. Chitosan

Over the last 30 years chitosan has gone through unimaginable development and has been successfully infiltrated into different areas of our lives. It is a natural product gained from chitin which is, after cellulose, the second more common biopolymer on earth (Majeti et al. 2000). Besides crayfish, molluscs and insects, chitin is also present in vegetal species, mainly in the cell-walls of fungi and moulds, where the nutritional cycle requires nitrogen (Table 1). Chitin structures are mainly derived from the ectoderm of multicellular animals and the typically built skeletons of invertebrates, while the collagen structures originate mainly from mesoderm cells (Table 2). Crayfish, molluscs, insects and fungi produce around 100 milliards tons of chitin a year, which is one of the least exploited sources of biomass on earth (Tharanathan 2003).

Type	Content of chitin [%]	Type	Content of chitin [%]
CRAYFISH		MOLLUSCA	
Crabs	72.1 ^c	Mussels (shell)	6.1
Crayfish	69.1 ^c	Oysters (shell)	3.6
Alaska crayfish	28.0 ^d	Squid (skeleton)	41.0
Lobster	60-75 ^c		
INSECTS		MOULDS, FUNGI	
Cockroaches (blatela)	18.4 ^d	aspergillus niger	42.0 ^e
Beetles	27-35 ^c	penicillium notatum	18.5 ^e
Flies (true flies)	54.8 ^c	penicillium crysogenum	20.1 ^e
Larva of silkworm (bombyx)	44.2 ^c	mucor rouxii	44.5 ^a
		Fungi (lactarius vellereus)	19.0 ^b

Table 1. The content of chitin in some of the organisms (Tharanathan 2003): ^aon the weight of the body; ^bon the dry weight of the body; ^con the weight of the organic part of the cuticle; ^d on the joint dry weight of the cuticle; ^eon the dry weight of the cell wall

Chitin is a polysaccharide, a combination of 2-acetamino-2-deoksi- β -D-glucosic units, connected with β -1,4 linkages, and chitosan is the common name for a large group of chitins,

deacetylated to different degrees. It is mainly a combination of 2-amino-2-deoksi-D-glycopyranose units, connected by β (1-4) linkages (Fig. 3).

The source of chitin	Proteins	Chitin	Ashes	Fats
Crabs (<i>Collinectes sapidus</i>)	25.1	13.5	58.6	2.1
Crabs (<i>Chionoecetes opilio</i>)	29.2	26.6	40.6	1.3
Crayfish (<i>Pandalus borealis</i>)	41.9	17.0	34.2	5.2
Crayfish (<i>Cragon cragon</i>)	40.6	17.8	27.5	9.9
Crayfish (<i>Penaeus monodon</i>)	47.4	40.4	23.0	1.3
Lobsters (<i>Procamborus clarkii</i>)	29.8	13.2	46.6	5.6
Common snipe	61.6	33.0	29.4	1.4

Table 2. The approximate composition of crayfish skeletons' offal in % according to the dry weight (Synowiecky & Al-Katheeb 2003).

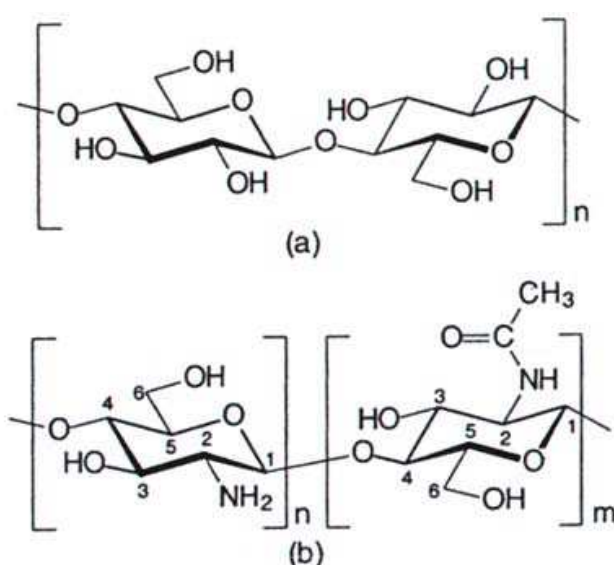


Fig. 3. Chemical structure of celluloses (a), chitin and chitosan (b) – chitin mainly consists of monomers in the shape of »m« (N-acetyl form), while chitosan, according to the degree of deacetylation, consists mainly of monomers in the shape of »n« (amine form) (Lim 2003).

Chitosan is a weak acid and, as such, is subject to reactions of neutralization in alkaline mediums. The free electron pairs on the primary amine group delegate chitosan as a potential nucleofil, which easily reacts with most aldehydes and forms imines. The molecules of chitosan have a highly positive polarity and attract negative molecules. Although most reactions with chitosan occur primarily on amino groups, it is also possible that hydroxyl groups selectively change. A hydroxyl group on the atom C6 is more reactive than the group of atom C3.

During the procedure of acquiring chitosan (Fig. 4), rough conditions usually lead to the degradation of products. The molecular mass of natural chitin is usually higher than one million, while the products of commercial chitosan have a molecular mass between 100, 000 and 1, 200, 000. According to the Horowitz method of deacetylation (Goosen 1997), which assumes a 30 minute treatment of chitosan at 180 °C, it is possible to gain chitosan with a 95% degree of deacetylation, but such a kind of chitosan has an average chain length of only

about twenty units. Generally speaking, factors such as the presence of oxygen, high temperature, and cutting charge can cause the degradation of a chitosan product.

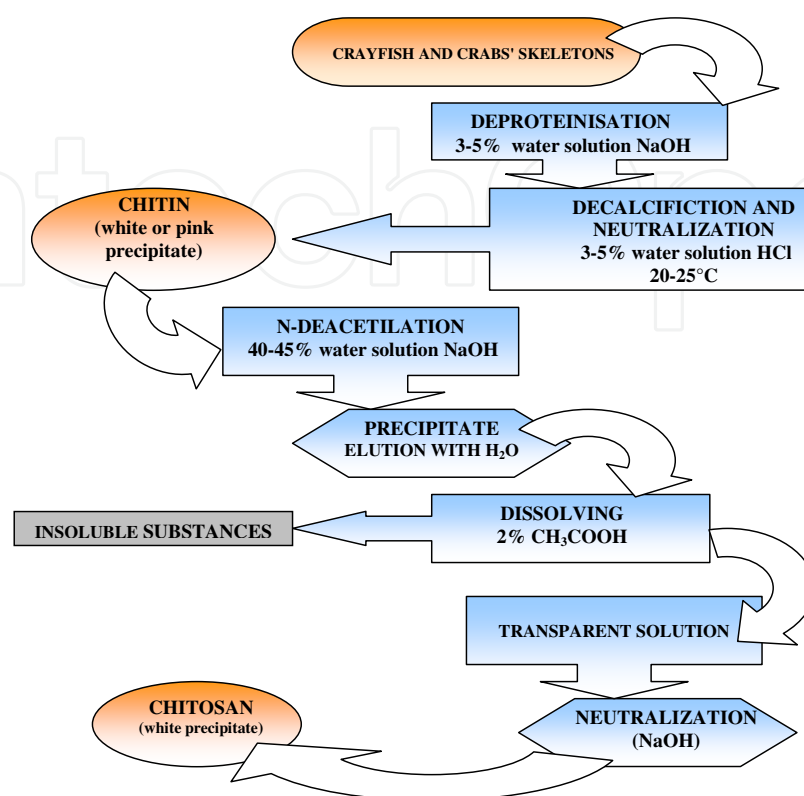


Fig. 4. The ways of chitin and chitosan acquisition from the skeletons of arthropods (Strnad et al. 2008)

The degree of deacetylation has a significant effect on the degree of chitosan dissolution in water solutions, since the chitosan with a degree of deacetylation of 40 % can be dissolved in water solutions to pH 9, while at 85 % degree of deacetylation it can be dissolved only in solutions to pH 6.5. Chitosan in the form of free amine is insoluble in water near its neutral pH, in concentrated acids' (except sulphuric acid) the bases, and organic solvents. On the other hand, chitosan is soluble in dilute HCl, HI, HBr, HNO₃ and HClO₄. There is a possibility that, in hydrogen acid after dissolution of the polymer, precipitation occurs in the form of a white-coloured gel. This biopolymer is also slightly soluble in dilute H₃PO₄ (Goosen 1997, Knnittel et al. 1998) The dissociation constant of chitosan is variable depending on the degree of dissociation at which it is determined. The Katchalsky equation is appropriate when calculating the variation of the pK_a value (Roberts, 1992). There are many factors that affect the viscosity of chitosan solutions, such as the degree of the polymer's deacetylation, the average, and the organisation of the molecular masses, concentration, ionic strength, pH value and temperature. The change in pH value of the polymer solution affects the viscosity of the solution differently depending on the acid used. The viscosity of the chitosan solution in an acidulous medium of ethan acid generally increases with any reduction in pH value of the solution, while the viscosity of the solutions in HCl with any reduction of pH value also reduces (Goosen 1997, Domzsy et al. 1983). Since the discovery of chitin, the use of chitin and chitosan has expanded over almost all fields of research (Muzzarelli 2005). Chitin and chitosan can be modified using different

chemical modifications e.g., nitration, acetylation, sulphonation, phosphorylation, etc (De Smedt et al. 2000). A broad spectre of derivates from chitosan can be gained using chemical modification which can then be used in various areas (Fig. 5).

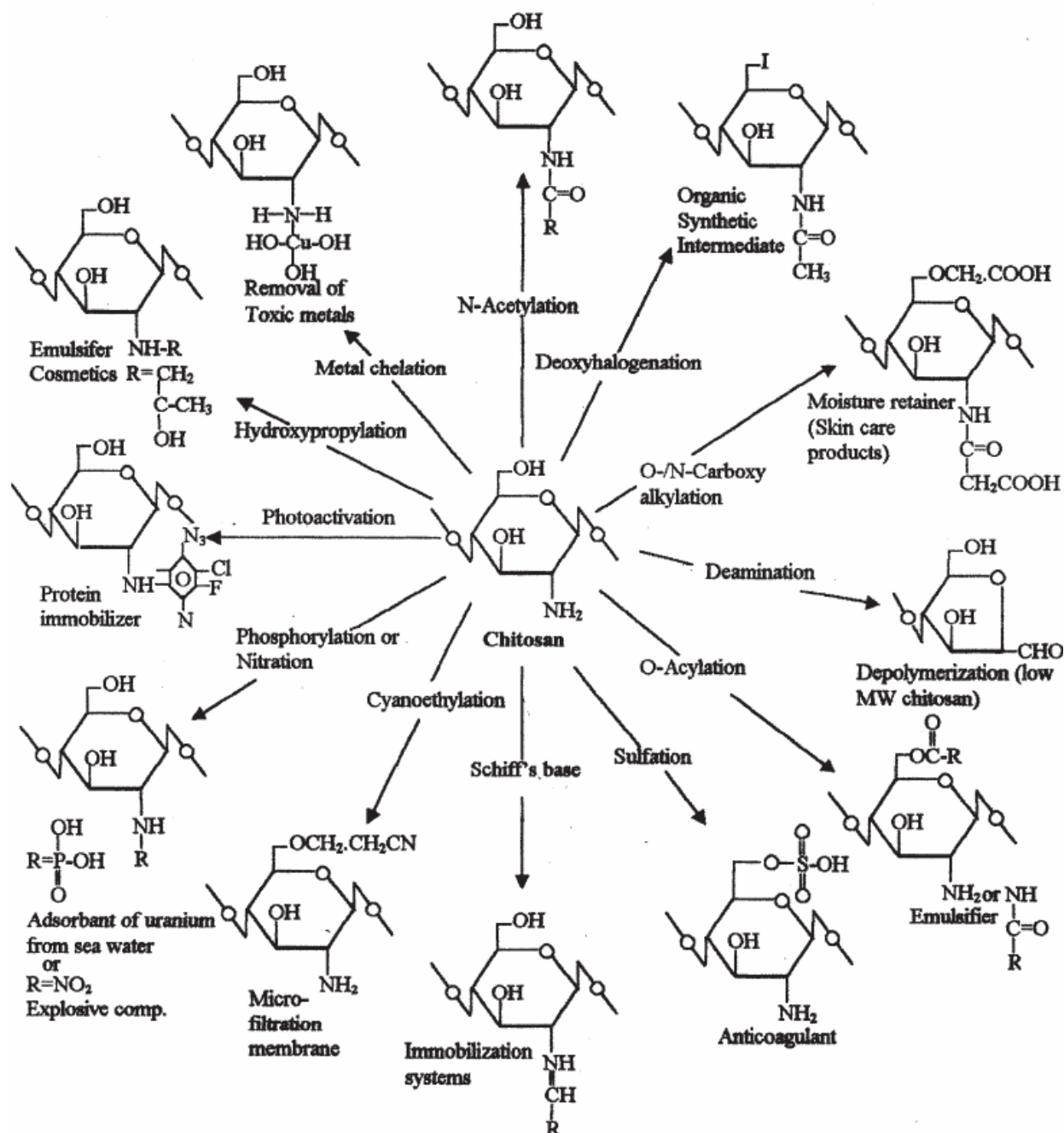


Fig. 5. Chitosan derivates and their potential use (Tharanathan 2003)

Biologically important characteristics have been found using some of these derivates, such as specifically immune, anticoagulant, spermicidal, fungistatic, bacteriostatic as well as flocculation properties (Rinaudo 2006, Smart et al. 2006, Chi et al. 2006, An et al. 2009, Kumar et al. 2010). As such chitosan derivatives can be used in ecology (for cleansing waste waters) as well as in nutritional, medical and pharmaceutical areas.

5. Cellulose fibres' surface functionalization

The use of natural cellulose fibres for developing medical materials has recently gained considerable attention, as emphasized by the numerous publications on that topic. Several methods to modify cellulose fibres for medical applications are described in literature: (i) oxidation procedures, (ii) synthesis of microbial cellulose, (iii) incorporation of metallic nanoparticles, and iv) various coating strategies at the finishing stages using quaternary ammonium compounds, PHMB (polyhexamethylene biguanides), triclosans, regenerable N-halamine and peroxyacid, some synthetic dyes, etc. (Edward et al. 2000, Browning 1967, Kaputskii et al. 2005, Kotel'nikova et al. 2003, Czaja et al. 2006, Hoenich 2006, Ravi 2000, Lim 2004). Besides the various fibrous, hydrogel (i.e. carboxymethyl cellulose) and alginate - based products, chitin and its derivate chitosan are currently the most popular as antimicrobial coatings for cellulose fibres. Chitosan offers many advantages over traditional cotton and regenerated cellulose fibres treatments because of its non-toxicity, biodegradability and biocompatibility (Fras Zemljic et al. 2009). Chitosan can be used for the introduction of active amino groups onto/into cellulose fibers, and in this way, for cellulose fibers' surface functionalisation. Similarities between the chemical and molecular structures of cellulose and chitosan enable high affinity between both polymers. The more possible cellulose-chitosan intermolecular interactions are based on H-bonds and Van der Waals forces, however, ionic and/or covalent bonds can be formed under special conditions and cellulose treatments.

The manipulation of chitosan binding strategies onto cellulose surfaces i.e. a) reversible binding, which enables the release of a chitosan from a fibre's surface and, b) irreversible binding resulting in the permanent bioactivity of a fibrous (textile) surface, enable the achieving of functional fiber surfaces for different applications.

5.1 Irreversible binding

For conventional textile applications, irreversible binding means that the substance-fiber binding is permeable to washing procedures. It is extremely important for irreversible attachment to introduce carboxyl or aldehyde groups into/onto the fibres which are then potential anchoring sites for chitosan molecules. In this way, electrostatic attraction is assured between cellulose fibres as host material, and chitosan as adsorbent. It has been confirmed that carboxyl groups in the cellulose have different effects on chitosan binding in comparison with aldehyde groups (Browning 1967).

In order to achieve permanent antimicrobial properties, the procedures for textile treatments using chitosan have to fulfill two rather contradictory conditions: 1) a satisfactory amount of anchoring sites in/on the fibers, onto which chitosan could permanently bind and, at the same time 2) a satisfactory amount of free amino groups of chitosan, which would be responsible for antimicrobial activity. The more appropriate activation procedures of cellulose fibres for permanent chitosan binding are those which introduce aldehyde groups. In this way, chitosan could be chemically attached onto cellulose. There are several procedures for obtaining aldehyde groups in cellulose, but the most useful are oxidation procedures using different specific oxidising agents (Browning 1967).

According to previous studies oxidation procedures with potassium or sodium iodatum (VII) (KIO_4) under special conditions lead to an increase in the cellulose reductive (aldehyde) group's amount on C2, C3 and C6 glycoside atoms (Browning 1967). There are also small amounts of aldehyde end-groups on C1, which can be further oxidized to

carboxyles (Fig. 6). The contents of the reducing groups in cellulose fibres increase with increasing oxidation time, and with KIO_4 solution concentration.

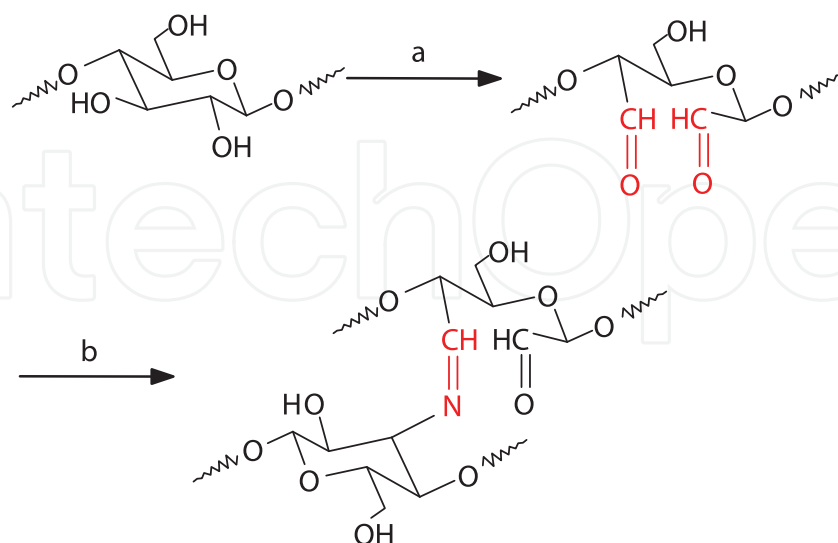


Fig. 6. Scheme of oxidation (a) and chitosan binding (b) onto cellulose

During the binding of chitosan onto oxidised fibres, the aldehyde groups on the fibres surface were reduced, due to the formation of Schiff's base between the chitosan and oxidised cellulose (Fras Zemljic et al. 2009). However, an increase of accessible amino groups was expected in cotton samples (non-oxidised and oxidised) coated with chitosan. A variety of techniques can be used for determination of accessible amino groups' amounts in cellulose fibres. One of them is spectroscopic determination of dye adsorption for C.I. Acid Orange 7 into cotton fibres when coated with chitosan, and the second very accurate method Polyelectrolyte titration and Acid Orange 7 methods showed that cotton samples previously activated by oxidation using potassium or sodium iodatum after chitosan treatment possessed lower amount of amino groups than non-activated fibres. This is expected since presence of aldehyde groups in fibres leads to the formation of electrostatic bonds with accessible amino groups of chitosan. Moreover, both techniques also showed that the amounts of the amino groups in the cotton sample, functionalised by a higher molecular weight of chitosan, were higher than in the cotton sample functionalised with low molecular weight chitosan. These results were in accordance with the zeta potentials of the fibres' surfaces which were calculated from measurements of streaming potential, as a function of pH (Fig. 7).

Pretreated cotton fibre showed typical $\text{ZP}=\text{f}(\text{pH})$ function for cotton, which was negative in practically the whole pH region, with a ZP plateau value -8 mV. The higher amount of aldehyde groups in the oxidised samples influenced the higher negative values of ZP in the plateau of these samples. After chitosan treatment, the functions $\zeta = \text{f}(\text{pH})$ showed typical amphoteric characteristics. At higher pH regions, the negative streaming potential is due to the dissociation of cotton carboxyl groups. Protonation of amino groups in acidic region causes the sign of the streaming potential to change into positive streaming potential at the isoelectric point.

After chitosan treatment the shifting of isoelectric point into higher pH region was observed. This is expectable since chitosan coatings of fibres lower the acidity of the cellulose fibre surface. On another hand, adsorbed chitosan compacts the fiber surface and thereby reduces

the hydration of the fibre interior (Čakara 2009) and consequently fibre swelling, which increase the negative ZP values in plateau values (see Figure 7).

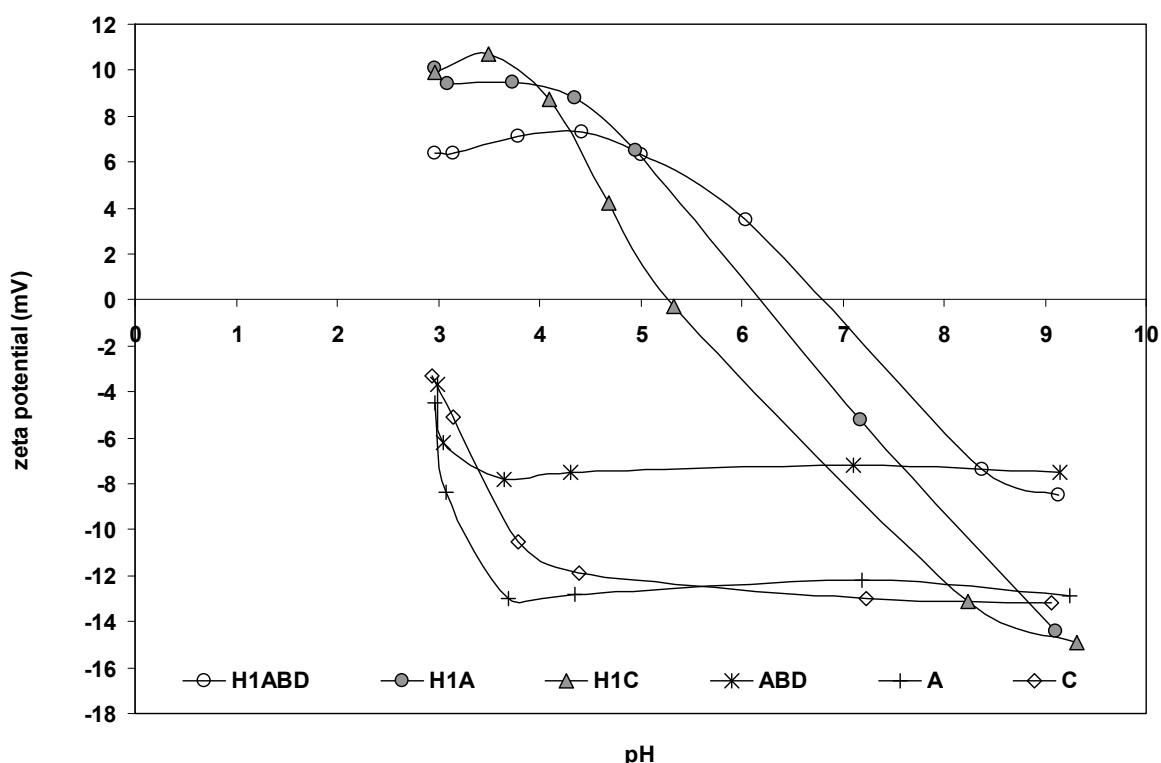


Fig. 7. Zeta potential (ζ) as a function of pH ($\zeta = f(\text{pH})$) for pretreated (ABD) and KIO_4 oxidized (A - 24 h, with 2,3 mg/mL KIO_4 and C - 72 h, with 2,3 mg/mL KIO_4) cellulose samples and the same samples, treated with chitosan H1

It was found out in the same research, that oxidation procedures using potassium iodatum had a large influence on fibres' mechanical properties. Even after a relatively short time of oxidation at room temperature the breaking force of the fibres decreased by about 34,5 % in comparison with non-oxidised samples (Strnad et al. 2008).

The second more appropriate way of permanent chitosan binding onto cellulose fibres are the reactions between chitosan amino groups and cellulose carboxyl groups. Several methods available in literature describe how cellulose fibres are modified in order to obtain carboxyl groups: oxidation procedures, adsorption of anionic polymers and dyes, plasma activation, etc. (Browning 1967, Kaputskii et al. 2006, Kotel'nikova et al. 2003, Fras Zemljič et al. 2006, Vesel et al. 2010).

In our previous research work, fibres were oxidized selectively as well as non-selectively with the aim of obtaining a different content of carboxyl groups (Fras Zemljič et al. 2004). The Methylene Blue method and Complexometric titration, which are based on the ion-exchange capacity of the cellulose, were used for determining the contents of the carboxyl groups. It was found that carboxyl groups' contents for oxidized fibers depend on the oxidation procedure conditions (concentration of oxidizing agent, time, pH, etc). Increasing the carboxyl groups' contents was more expressive using a non-selective oxidation in comparison to selective oxidation, where the oxidation mechanism is known. During non-selective oxidation, any possible oxidation products may occur because the course of the oxidation process is unpredictable. A mixture of products is present oxidized to different

degrees and according to different mechanisms. The DP (degree of polymerization) of cellulose fibres was drastically reduced. On the basis of extensive studies it was established that, due to extreme worsening of fibre mechanical properties, oxidation is an inappropriate tool for fibre activation. It has become clear that different standard chemical processes such as oxidation procedures usually modify the fibres both structurally and chemically and, in addition to frequently being ecologically less desirable, are detrimental to the fibres' mechanical properties (Liu 2007).

Therefore, over the last decade, the tendency of fibre functionalisation has been aimed at using natural, biodegradable, and non-aggressive chemicals. Polysaccharides are currently the most promising among them as antimicrobial coatings for cellulose fibres (Dimitriu 2002).

It has been shown that modification of fibres by the adsorption of carboxymethyl cellulose (CMC) introduces new carboxyl groups onto the fibres' surfaces. The increase of charge was determined indirectly by phenol-sulphuric acid method, and directly by conductometric titration. It was discovered that the total charge of cotton increased by more than 50 % in the case of all used CMC products. Even more, the modified fibres appeared to have better mechanical properties (Fras Zemljič et al. 2006) (Table 3).

Sample	Titer dtex	Tenacity cN/tex	Elongation %	Force cN	Young modulus cN/tex
Non-modified fibres	1.84	31.94	11.61	5.66	105
CMC-modified fibers	1.94	33.24	13.57	6.39	82

Table 3. Mechanical properties of CMC-functionalized fibres

The titer and tenacity of CMC-modified fibers can be increased by about 5 %, while with respect to elongation and force, about a 15 % increase is obtained in comparison with non-modified fibers (reference sample). Moreover, because of the capacity of cotton fibres to adsorb cationic surfactants, the rate of adsorption is increased by the previously adsorbed carboxymethyl cellulose (CMC) on the fibres' surfaces (Fras Zemljič et al. 2006), see Fig. 8.

The adsorption of cationic surfactant onto fibres increases as the anionic charge on the fibres increases (due to CMC adsorption). Due to the attraction between surfactant cations and dissociated fibre carboxyl groups, a higher charge density on the fibres leads to a higher and faster adsorption of surfactant (Fras Zemljič et al. 2006).

This procedure of fibre anionization may be due to extremely anionic charge increase and improved mechanical fibre properties at the same time, very promising for irreversible chitosan binding. This work is presently in progress.

Another possibility of introducing carboxyle groups into cotton fibres is treatment with 1, 2, 3, 4 buthanetetracarboxylic acid (BTCA) (El-ahlavy 2005). Our research results showed that an adequate number of free carboxyl groups can be assured with the treatment of cellulose fibres by this anionic crosslinking agent. BTCA treatment played an important role in chitosan fixation and chitosan treatment durability. The amount of carboxyl groups introduced into cellulose fibers strongly depends on the BTCA concentration in the impregnation solution. In combination with an appropriate catalyst (e.g. $\text{NaH}_2\text{PO}_2 \cdot \text{H}_2\text{O}$) a satisfactory amount of accessible carboxyl groups is achieved. The carboxyl groups' amounts in the BTCA treated samples may be determined using the Sobue-Okubo modified complexometric titration method (Fras Zemljič 2004). The results of complexometric titrations showed that after treatment with 7 % BTCA solution, the cellulose contained a

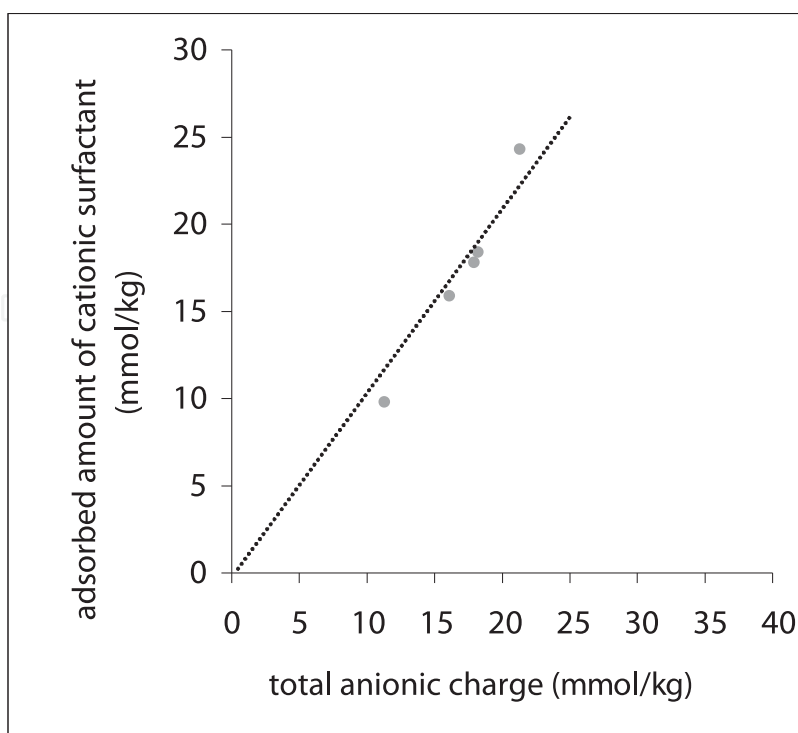


Fig. 8. The adsorbed amount of cationic surfactant (determined by spectroscopy) as a function of the total charge of the fibres, as determined by conductometric titration.

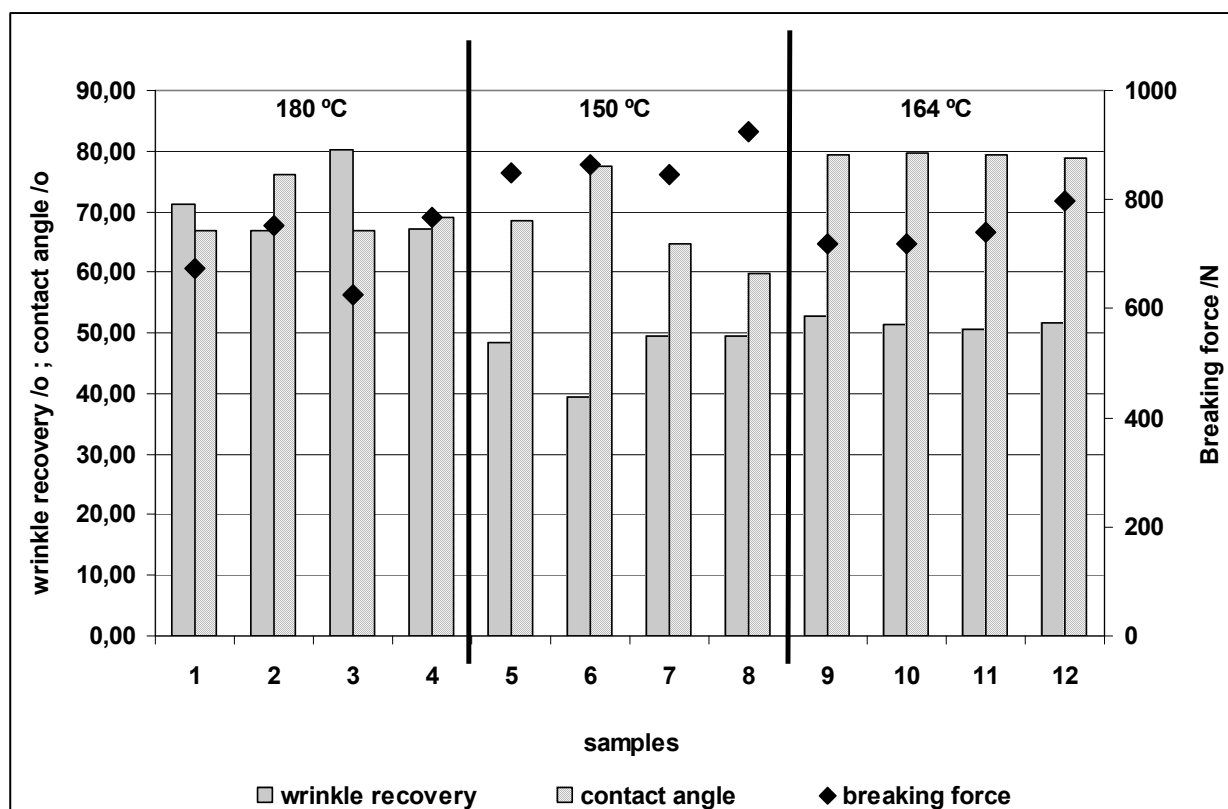


Fig. 9. The influence of process conditions on the wrinkle recovery, contact angles and breaking force of BTCA and chitosan treated cotton fibers

higher amount of carboxyl groups when compared to the 3 % BTCA solution. The BTCA treatment, on the other hand, improved the mechanical properties of the samples (Fig. 9). Chitosan was adsorbed onto previously BTCA-treated fibres, which gave the same tendency regarding results as in the case of oxydised fibres i.e. reduction of the amounts of accessible amino groups on the chitosan treated fibers due to interactions with carboxyle groups of fibres. However, it has been discovered, that BTCA- treated samples, showed in comparison with non treated material, a much better washing resistency of chitosan.

Gas plasma treatment of fibres is the next possibility of cellulose fibre surface activation for later chitosan adsorption. Cold plasma treatment is an extremely versatile technique for modifying the polymer surfaces of totally different shapes (Clark 1979 et al., Wertheimer et al. 1996, Kunaver et al. 2004, Mozetic 2004, Morales et al. 2006, Sharnina et al. 1996, Tu et al. 1994, Prabaharan et al. 2005). It has been reported that plasma treatment can improve polymer-polymer adhesion (Clark et al. 1979, Wertheimer et al. 1996), the best results being obtained when using oxygen plasma (Carlsson 1991, Felix et al. 1994, Couto et al. 2002). The purpose of our investigation (Fras Zemljič et al. 2008) was evaluating to what extent oxygen plasma treatment could improve the chitosan adsorption, and in this way, the antimicrobial activities of chitosan covered fabrics. The result of surface activation using oxygen plasma is the formation of different oxygen-containing polar functional groups such as C-O, C=O, and O-C=O, which act as nucleophilic centres to which adsorbent atoms can bind. The influence of low pressure oxygen plasma treatment on the functionalization of cellulose material using chitosan was investigated by conductometric titration and XPS. The results were supported by conventional Kjeldahl analysis. The effect of plasma treatment on the antimicrobial capacity of chitosan treated fabrics was studied according to the ASTM E2149-01), which is a quantitative antimicrobial test method performed under dynamic contact conditions (Fras Zemljič et al. 2008). Both conductometric titration and XPS indicated that chitosan adsorbed onto the viscose fabric, irrespective of whether it had been activated with plasma or not. However, the amounts adsorbed on plasma-treated samples were significantly higher than those on non-treated samples (Fig. 10), and increased with increasing chitosan concentration in the solution used for treatment.

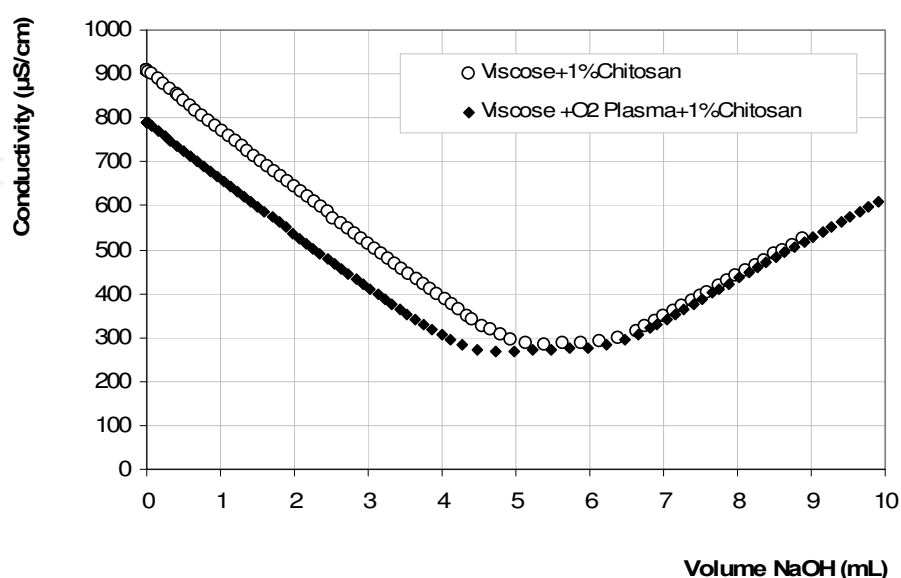


Fig. 10. Conductometric titration curve of viscose fibers (activated and non-activated by oxygen plasma), modified with 1 % chitosan solution

Increasing the number of active functional groups due to plasma activation in the cellulose surface enabled the adsorption of higher amounts of chitosan and, consequently, a higher amount of amino groups responsible for antimicrobial activity (Lim 2004, Ravi Kumar 2000, Liu et al. 2007, Zitao et al. 2003). The higher amount of amino groups in plasma-activated-chitosan-treated samples increased the probability that a protonated amino group met the bioplasm of the bacteria and, in this way, resulted in a greater bacterial reduction capacity (Zitao et al. 2003).

Sample treatment	R, %				
	Pathogenic bacteria			Pathogenic fungi	
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Streptococcus agalactiae</i>	<i>Candida albicans</i>	<i>Candida glabrata</i>
Non-treated viscose fabric	0	0	99	20	34
Plasma treated fabric (24 h after treatment)	0	0	80	51	21
Non-treated fabric impregnated by 1 % chitosan solution for 24 h	70	0	100	0	98
Plasma treated fabric impregnated by 1 % chitosan solution for 24 h	91	0	100	100	100

Table 3. Antimicrobial properties of fibres impregnated by chitosan; plasma pre-activated and without plasma pre-activation

Table 3 shows that the plasma activation for sample treated using 1 % of chitosan solution, affected the degree of bacterial reduction R . R for *Staphylococcus aureus* increased from 70 % to 91 % and for *Candida Glabrata* from 98 % to 100 %. Even more, the plasma-activated-chitosan-treated samples showed a total reduction in *Candida Albicans*, for which, in the case of the non-activated sample $R = 0$ % (Fras Zemljic et al. 2008).

It has to be pointed out that several past researches by our group showed that, in some cases, when chitosan interacts with non-activated cellulose, adsorption could also be irreversible. This is somehow expected due to the fact that, cotton fibres especially are weakly acidic due to pre-treatments such as scouring and bleaching (Fras Zemljic et al. 2008) and cationic polymers may be readily adsorbed onto fibres by electrostatic attraction. However, Ćakara et al. 2010 showed that irreversible adsorption of chitosan onto weakly acidic cotton fabric is, under present conditions, predominately driven by a non-electrostatic attraction. Myllitye et al. 2009 evidenced a non-electrostatic interaction between chitosan and cellulose at low pH. This may be attributed to specific structural interaction between chitosan and cellulose (H-bonds and hydrophobic interactions). In the same way, several neutral or even anionic polysaccharides, including xyloglucan and carboxymethyl cellulose are irreversibly adsorbed onto cellulosic fibres as well (Laine et al. 1994).

For cellulose fibres functionalised by chitosan (irreversible binding), the charging isotherms exhibit a charge reversal around $\text{pH} \approx 6$, which is identified as the point of zero charge (PZC). These fibres protonate according to the one-pK model with two determined pK values such as $\text{pK}_{(\text{chitosan})} = 6.3$ and $\text{pK}_{(\text{acidic cotton groups})} = 4.7$. pK is not only important for classifying acid strength but also determines the properties of a substance in nature or its possible use as a drug. Determination of the pK value is therefore of great importance for biomedical

applications as, for example, medical textiles in wound healing, gynaecological treatment, etc. (Čakara 2009).

5.2 Reversible binding of chitosan

Chitosan solubility is pH-dependent, therefore, changes in pH could be applied for a tuning of adsorption/desorption of chitosan by changes from soluble to insoluble forms of it.

One of the latest researches of our group is based on comparing the adsorption of totally soluble chitosan (from acidic solution) against the adsorption of precipitated chitosan, onto cellulose fibres. This topic was investigated by ATR-FTIR, potentiometric titration, and the conventional spectrophotometric method using C.I. Acid Orange 7 adsorption. The binding of precipitated chitosan leads to a min. 70 % of chitosan desorption. However, the functionalization of viscose fibres using precipitated chitosan is, in a contrast to the functionalization of fibres with acidic chitosan solution, more efficient in the sense of active (accessible) amino groups' amounts and, in this way, in antimicrobial activity.

Cellulose fibres, treated using chitosan precipitation showed a slightly increased reduction of pathogenic bacteria (*S. aureus*) and fungus (*C. albicans*) in comparison with the fibres treated by the adsorption of totally dissolved chitosan from acidic solution.

The most popular approach for reversible chitosan/fibre binding is currently the preparation of chitosan micro/nanoparticles. These can act as an antimicrobial agent itself or as drug carrier in delivery systems. There are several ways for synthesising chitosan nanoparticles, such as emulsification, oppositely-charged polymers' precipitation, the ionic gelation process etc. (Wan Ajun et al. 2008).

Chitosan nanoparticles showed high cytotoxic activity toward tumor cells, while low toxicity against normal human liver cells (L-02) (Qi, et al. 2005). Furthermore, chitosan nanoparticles showed high sorption capacity and anti-bacterial activity (Qi & Xu, 2004). The unique cationic character of chitosan nanoparticles could provide higher affinity to negatively-charged biological membranes and site-specific targeting in vivo (Qi et al., 2004). Particle size had an substantial influence on increasing their anti-tumor efficacy when chitosan nanoparticles are applied by intravenous injection (Qi et al., 2005). The unique character of the positive charge and small particle sizes of chitosan nanoparticles is responsible for their in vivo efficacy (Qi & Xu 2006).

If certain preparatory conditions are used, the resulting nanoparticles prove to be highly effective for providing new functionalities to the host material, such as with cellulose fibres. The treatment of cotton with poly(n-butyl acrylate) cores and chitosan particles confers the fabric with excellent antibacterial property. Other characteristics of particle-coated fabrics including mechanical properties, air permeability, hand feel and antibacterial durability over repeated launderings are reported as well. Using chitosan microcapsules/nanocapsules containing several extracts oil were performed to introduce antimicrobial properties. Moreover, the pleasant fragrance of aromatic oils was retained on fibre surfaces at least six months (Alonso et al. 2010, Ye et al. 2005). It has been shown that cellulose coated by chitosan nanoparticles describe a cost effective and non-toxic methodology for new textiles developing. There are several possible interactive mechanisms (Fig. 11) between cellulose and chitosan nanoparticles.

In the future, this work will have implications in the design of medical textiles adsorbed by vehicles for the loading of several drugs as targeted drug delivery, protection from enzymatic degradation, and reduced drug toxicity or side effects. This may present therapy for solving several skin and several other mucous membranes problems (vaginal, mouth, etc).

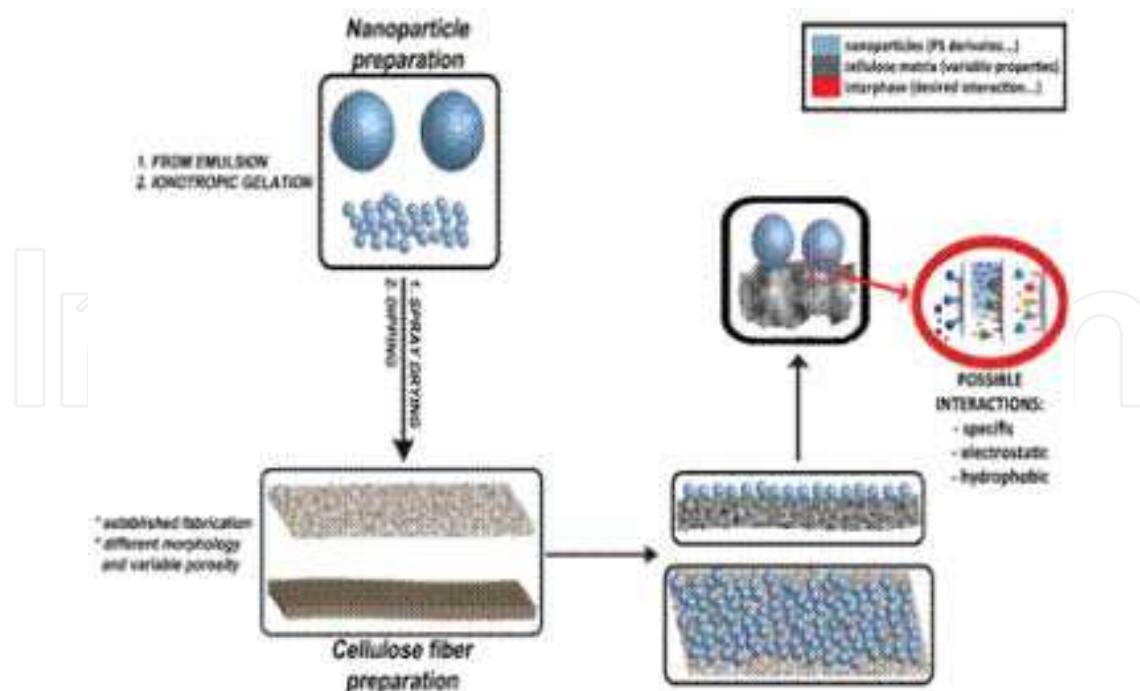


Fig. 11. Possible interaction mechanisms between cellulose and chitosan nanoparticles

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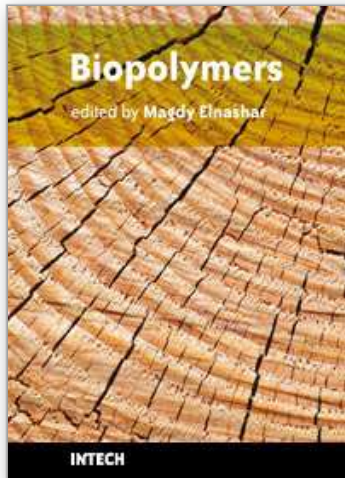
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