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Enzyme-assisted modification of cellulose/chitin fibers with NIPAAm

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Abstract: Coating processes are applied in order to improve coating adhesion and resistance to degradation. Covalently bound organic coatings rather than merely physically bound ones assure stable modification. In this study a novel two-step process was developed to modify cellulose/chitin mix fibers consisting of enzymatic activation with a commercial cellulase, followed by a coupling reaction with N-isopropylacrylamide (or poly (N-isopropylacrylamide)) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and N-hydroxysuccinimide (NHS).

Both enzyme-activated and subsequently modified samples were characterized by ATR-FTIR, XPS, and SEM. All obtained results confirm the structural and morphological changes of the fiber surface after the application of the two-step procedure. The particular responsiveness to temperature and to pH of the coated fibers was evidenced by following the swelling behavior. It was established that the swelling kinetics followed a Fickian behavior.

Key words: Fibers, responsive, cellulose, chitin, enzymes

1. Introduction

Textile fibers have demonstrated their utility in many fields, but many of them do not have special characteristics needed for certain applications without further treatments. The textile industry is searching for innovative production techniques to improve product quality, and society also requires new finishing techniques working with respect for the environment.

Of the many kinds of polysaccharides, cellulose and chitin are the most important biomass resources.[1] As is generally known, cellulose fibers are moisture-absorbent and comfortable, while chitin fibers are biostatic, inflammation diminishing, odor-resistant, odor-preventing, and itch-resistant.[2] Therefore, the combination of cellulose with chitin is an attractive task for many researchers and companies to produce materials with combined and/or special properties.[3,4]

Pretreatment of the cellulosic materials can be based on various techniques by using enzymes,[5,6] physical or chemical methods,[7,8] dissolution, fractionation[9] etc. The effect of the pretreatments can be followed by dissolution behavior and reactivity of the fibers in the subsequently applied modifications. The enzymatic activation of synthetic and natural materials has provided several advantages and a new strategy for obtaining useful polymers under mild reaction conditions with regard to temperature, pressure, and pH, and the use

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of nontoxic reagents,[10,11] being an environmentally friendly process, which can be a good example of "green polymer chemistry".[12]

Cellulases are responsible for the hydrolysis of the β-1,4 glucosidic bonds in cellulose. They are members of the glycoside hydrolase families of enzymes, which hydrolyze oligosaccharides and/or polysaccharides. Cellulase acts on the cellulose component, creating new active centers such as aldehyde groups in glucose, which can react with many functional groups such as amino and phenols in relatively mild conditions. Other groups from cellulose and chitin are also reactive.[13]

Scientists have made many attempts to develop smart textiles by graft copolymerization of environment-responsive polymers (ERP) onto the surface of fabrics.[14,15] Among the ERPs used for this purpose, poly (N-isopropylacrylamide) (PNIPAAm), as a temperature-sensitive polymer, is one of the most intensively investigated. It has simultaneously a hydrophilic and hydrophobic structure, and demonstrates a low critical solution temperature (LCST) at about 32 °C, which is close to body temperature. In an aqueous solution, the macromolecular chains of PNIPAAm experience reversible solubility and exhibit a significant hydration–dehydration change in response to temperature stimulus.[16] PNIPAAm and its copolymers and hydrogels can be applied to obtain temperature-sensitive hygroscopic fabrics, environment-sensitive deodorant fibers, and stimuli-sensitive nutrient delivery fabrics. When such materials are exposed to external stimuli, they display swelling/shrinkage or hydration/dehydration properties, and cause changes in the water vapor transmission rates and permeability of fabrics. It has been shown that initiators are used in the case of a N-isopropyl amide (NIPAAm) polymerization reaction for two reasons: (a) to initiate the polymerization reaction and (b) to maintain and ensure stability.[17]

In our previous work, in order to obtain stimuli-responsive fibers, cold plasma activation followed by reaction with NIPAAm and PNIPAAm was applied to modify cellulose/chitin (CC) mix fibers.[18,19]

This paper deals with the enzyme activations of CC mix fibers followed by the reaction with NIPAAm in order to establish the optimal conditions for the fiber's surface modification. The main aim of the study was to obtain new fibers with special properties. A commercial cellulase enzyme was used to activate the fiber surface by hydrolytic and oxidative/reductive reactions, respectively, for increasing the efficiency of the subsequently applied coating reaction with NIPAAm. Surface properties of the substrates were characterized by the powerful techniques of surface and structure analysis, such as ATR-FTIR, XPS, and SEM. Furthermore, changes in bulk properties were also evaluated by the swelling test.

2. Results and discussion

2.1. Elemental analysis

From elemental analysis (Table 1), it was found that the content of nitrogen was around 0.80 wt%, which indicates an 11.6 wt% chitin content in Chitcel, close to that given in the provider's specifications. The percentage of nitrogen increases for enzyme-activated and NIPAAm-treated samples, proving their its presence in Chitcel. Based on the nitrogen increased content of the NIPAAm-treated samples, a modification degree of the entire mass can be estimated at around 3.3% for cellulase-activated fibers.

Table 1. Elemental analysis results.

Sample	% C	% H	% N
CC	41.02	7.05	0.80
CC/cellulase/NIPAAm	41.65	6.61	1.21

2.2. ATR-FT-IR spectra of the modified fibers

The spectra of the fibers, shown in Figure 1, contain the main infrared spectral bands of the cellulose and chitin as components of the mix fibers and also bands appearing after enzymatic activation and consequently modification with N-isopropylacrylamide. By enzymatic degradation of the cellulose component, more chitin can emerge in the surface layer.

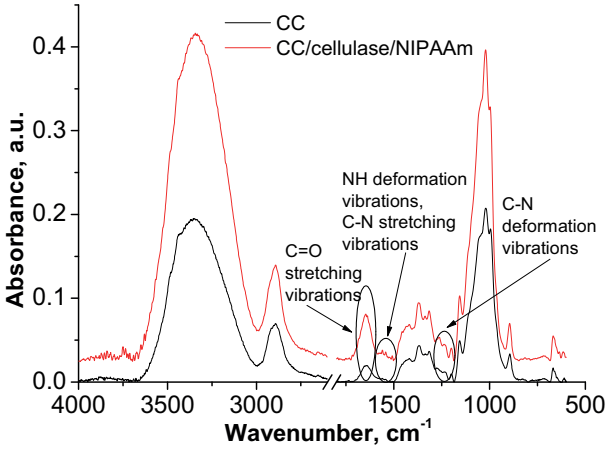

Figure 1. FTIR spectra of cellulose/chitin mix fibers and enzyme-activated NIPAAm-modified fibers.

Two information-rich regions of the FTIR spectra, namely 3700–2700 cm^{-1} and 1800–600 cm^{-1}, were examined. Table 1 shows the most significant absorbance bands in the 3700–600 cm^{-1} region and their assignments for CC fibers modified with NIPAAm. The main bands were assigned according to the literature data.[20] The infrared bands between 3700 and 3000 cm^{-1} correspond to the OH stretching frequencies of cellulose and the bands between 3000 and 2700 cm^{-1} correspond to different stretching vibrations of the CH group, while the bands from the 1800–600 cm^{-1} region, also called the "fingerprint region", correspond to different stretching or bending vibrations of the functional groups of the cellulose and chitin fiber components.

Some new bands appear in the FTIR spectra of NIPAAm-modified fibers (Figure 1; Table 2) that correspond to NIPAAm: 1558, 1542 cm^{-1} (assigned to CONHR group – NH deformation vibrations, C–N stretching vibrations); or to the new links that were formed between CC mix fibers and NIPAAm: 1646 cm^{-1} (assigned to $CONR_2$ group, C=O stretching vibrations), 1508 cm^{-1} (assigned to R_2NH group, NH deformation vibrations), and 1237 cm^{-1} (assigned to alkyl –N–$(alkyl)_2$ group, C–N stretching vibrations).

Table 2. Position and assignments of bands from FTIR spectra of untreated and NIPAAm-modified fibers.

Wavenumber (cm^{-1})	Band assignment and comments	CC	CC/cellulase/NIPAAm
3375–3340	O3–H3–O5 intramolecular H bond	3354	3343
2910–2850	symmetric CH_2, stretching vibrations	2892	2892
1650	C=O stretching vibrations (Amide I)	-	1646
1550	NH deformation vibrations C–N stretching vibrations	-	1558, 1542
1580–1490	NH deformation vibrations	-	1508
1460	CH_3 asymmetric, deformation vibrations	1457	1456
1430–1406	H–O–C in plane bond	1419	1435, 1420
1275–1000	C–O–C stretching vibrations	1276	1275, 1263
1280–1180	$C_{aliphatic}$–N, stretching vibrations	-	1237

Additionally, there were no bands assigned to the double bond C=C in the spectra of the cellulase activated fibers, indicating that NIPAAm either bonded or polymerized with enzyme treatment. There are also important shifts in the bands' position to lower or higher wavenumbers, or splits of some bands (Table 2), confirming the formation of new compounds after the two-step treatment. Based on these results, it can be concluded that the modification took place after enzyme activation.

The following spectral characteristics were evaluated:

- The energy of the H-bonds was calculated using Eq. (1):[21]

$$E_H(kJ) = 1/k[(\nu_o - \nu)/\nu_o], \tag{1}$$

where ν_o-standard frequency corresponding to free –OH groups (3650 cm^{-1});

$\nu-$ the frequency of the bonded –OH groups; k = 4 $\times$ 10^{-3} kJ^{-1}

- The enthalpy of H-bond formation was evaluated using Eq. (2):[22]

$$\Delta H(kJ/mole) = 0.0672\Delta\nu_{OH} + 2.646, \tag{2}$$

where $\Delta\nu_{OH}$ - OH wavenumber shift (cm^{-1})

- The H-bonding distance(R) (Å) is obtained by the Sederholm Eq. (3):[23]

$$\Delta\nu(cm^{-1}) = 4.43 \times 10^3(2.84 - R), \tag{3}$$

where $\Delta\nu = (\nu_o - \nu)/\nu_o$ – OH monomeric stretching frequency of 3600 cm^{-1};[24]

$\nu-$ OH stretching frequency in the sample IR spectrum

- The relative value of the optical density (RVOD) from the FT-IR spectrum was calculated by Eq. (4):[25]

$$RVOD = E_C(\nu_j)/E_C(\nu_s), \tag{4}$$

where E_C $(\nu j) = \lg T_C$ $(\nu_j)/T$ (ν_j)

$T(\nu_j)$ transmission at ν_j frequency when component content is 0;

$T_C(\nu_j)$ – transmission at ν_j frequency;

ν_s – standard frequency of 1425 cm^{-1}

- The asymmetric index (a/b) is the ratio between peak full width at half height of the OH absorption band.[26]

The obtained data are summarized in Table 3.

After modification/grafting an increase in the asymmetric index from 0.75 to 0.83, an increase in the hydrogen bonding energy from 20.3 kJ to 20.9 kJ, and a decrease in the hydrogen bond enthalpy from 47.5 kJ/mol to 47.1 for CC/NIPAAm–cellulase were obtained, showing that the structural order had been modified (Table 3).

Table 3. Spectral characteristics of NIPAAm-modified fibers in comparison with the untreated CC fibers.

Sample		CC	CC/cellulase/NIPAAm
OH band position (cm^{-1})		3354	3343
a/b ratio		0.75	0.83
E_H (kJ)		20.27	20.96
ΔH (kJ/mol)		47.45	47.06
R (Å)		2.78	2.78
χ_{IR} (%)	A_{1370}/A_{2900}	0.65	0.61
	A_{1420}/A_{900}	0.99	0.97
A_{OH}/A_{CH} ratio	A_{1315}/A_{1370}	0.94	0.86
	A_{1315}/A_{2900}	0.54	0.52

The low values of the A_{1370}/A_{2900} and A_{1420}/A_{900} absorbance ratios, which are proportional with the values of the crystallinity index, and the decrease in the A_{OH}/A_{CH} ratio indicate that the treated samples show a very disordered structure caused by the newly formed links. Based on the differences in the spectral characteristics, it can be concluded that cellulase enzyme was an efficient activator of the CC mix fibers.

2.3. XPS results

XPS provides quantitative information on the chemical composition of the surface and the differently bonded carbon atoms as well. For CC mix fibers, the carbon and oxygen peaks include information on the different functional groups that could be gathered from the high-resolution spectrum.

The relative composition in O and C atoms on the surface and the calculated oxygen to carbon ratio (O/C) for all species are listed in Table 4. The data presented show that the percentage of oxygen detected was higher on the surface of CC/NIPAAm fibers (32.6%) than on that of the untreated ones (27.9%), while the percentage of carbon atoms was lower on the surface of all CC-modified fibers (60.1%) than on that of the untreated ones (72.1%).

Table 4. Binding energy and relative concentration for untreated and enzyme-treated fibers.

Element		C1s	O1s	O/C ratio	N1s
CC	Binding energy (eV)	285.50	532.50	-	-
	Area (atomic %)	72.1	27.9	0.38	-
CC/cellulase/NIPAAm	Binding energy (eV)	285.99	532.01	-	399.49
	Area (atomic %)	60.1	32.6	0.54	7.3

After modification, the O/C ratio increased for CC/NIPAAm enzyme-treated fibers, suggesting that surface modification occurred. No amount of nitrogen was found on the surface of untreated CC mix fibers. Nitrogen can be detected only on the surface of the enzyme activated fibers (1.3%) and on the subsequently treated samples (7.3%).

The most evident change in the fiber structure, or at least on the surface of the fiber, can be detected in the number and type of carbon atoms. High resolution C1s spectra of both untreated and enzyme-treated fibers were deconvoluted and showed four types of carbon atoms (Table 5), namely C1 - carbon atom bonded only to another carbon (C–C) or to a hydrogen atom (C–H); C2 - carbon atom bonded to an oxygen atom (C–O from C–OH), to a nitrogen atom (C–N), or from C–O–C group; C3 - carbon atom single bonded to two oxygen atoms (–O–C–O–) or to a single carbonyl atom (–C=O) or a single bonded to a nitrogen atom and double bond

to an oxygen atom (N–C=O); C4 - carbon atom single bonded to an oxygen atom and to a carbonyl oxygen atom (O–C=O).

Table 5. Binding energy and relative concentration of C1s peak for untreated and enzyme-treated cellulose/chitin mix fibers.

Carbon type		C1	C2	C3	C4	C_{ox}/C_{unox}
CC	Binding energy (eV)	284.6	286.0	287.0	288.9	-
	Area (atomic%)	48.59	32.69	17.44	1.28	1.06
CC/cellulase/NIPAAm	Binding energy (eV)	284.4	285.9	287.3	290.2	-
	Area (atomic%)	21.22	34.59	39.25	4.94	3.71

After modification, no significant changes in binding energies were observed. For all samples, after modification the percentage of the C1 peak decreased and of the C2 peak increased. A possible explanation is that through the modification process new links are formed. Thus, the inner layers of the fibers, which contain chitin, are revealed and provide C–N links. Furthermore, the C3 and C4 peaks showed an increase for NIPAAm modified samples. The C4 peak was a small percentage in all samples, most likely due to the low concentration of carboxylic groups on the surface of the fibers.

The hydroxyl group content in the superficial layers of the cellulose/chitin mix fibers is 32.69%. Moreover, the C2 groups (C–O– from C–OH) increase to 34.59% for CC/NIPAAm obtained by activation with cellulase. C3 groups also increase significantly from 17.44% to 39.25% for CC/NIPAAm obtained by activation with cellulase.

Based on the above values, a modification degree of the superficial layers can be estimated, and the value obtained was 47.29%.

In addition, the oxygenated to unoxygenated carbon ratio (C_{ox}/C_{unox}) was also calculated following Eq. (1):

$$\frac{C_{ox}}{C_{unox}} = \frac{C_{oxygenated}}{C_{unoxygenated}} = \frac{C_2 + C_3 + C_4}{C_1} \tag{5}$$

The XPS data listed in Table 5 show an increase in the total oxygenated carbon bond, the C_{ox}/C_{unox}, also suggesting that surface modification occurred.

The oxygen deconvoluted peaks evidenced two types of oxygen atoms (Table 6): O1: oxygen atom linked to a carbon atom by single bond (C–O–) or a hydroxyl oxygen (OH); O2: oxygen atom bonded to a single carbonyl atom (–C=O), single bonded of two carbon atoms (C–O–C), or a hydroxyl oxygen atom bonded to a carbon atom (C–OH).

Table 6. Binding energy and relative concentration of O1s peak and N1s peak for untreated and enzyme activated and NIPAAm-modified cellulose/chitin mix fibers.

Oxygen type		O1	O2	N1	N2
CC	Binding energy (eV)	532.4	534.8	-	-
	Area (atomic%)	98.82	1.18		-
CC/cellulase/NIPAAm	Binding energy (eV)	532.3	534.0	399.8	401.9
	Area (atomic%)	68.60	31.40	74.30	25.70

The O1 peak is assigned to an oxygen atom linked by a single bond to a carbon atom or a hydroxyl oxygen, and showed a decrease after enzyme modification. At the same time, following modification, the O2

peak, which is characterized by a higher binding energy, showed an increase caused by the new links created by enzyme treatment.

Nitrogen was found only on the surface of the modified samples. N1s peaks were deconvoluted and showed two types of nitrogen atoms for enzyme-treated samples (Table 6): N1: nitrogen atom linked by a single bond to two carbon atoms (C–NH–C) or a nitrogen atom linked to a single carbonyl atom (N–C=O); N2: nitrogen atom linked to a carbon atom by a single bond (C–N).

The value of the N1 peak, assigned to a nitrogen atom linked by a single bond to two carbon atoms or to a nitrogen atom linked to a single carbonyl atom, is higher than the value of the N2 peak, corresponding to a nitrogen atom linked to a carbon atom by a single bond for NIPAAm treated fibers.

Based on ATR-FTIR and XPS results, we can conclude that NIPAAm polymerized using EDC and NHS as polymerization reaction initiators and the reaction between CC fibers and NIPAAm took place successfully. Figure 2 presents possible routes for enzymatic modification on CC mix fibers.

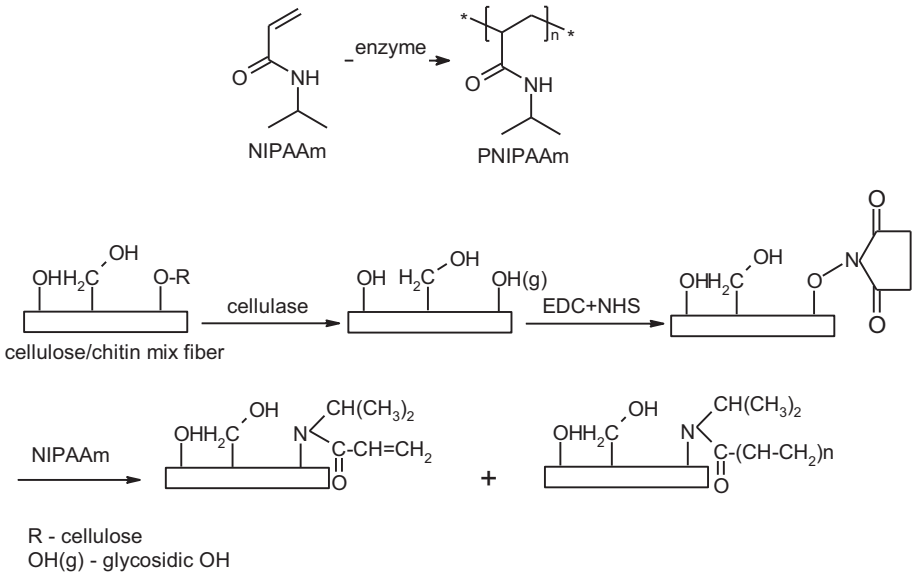

Figure 2. Schematic enzymatic modification of cellulose/chitin mix fibers with NIPAAm.

The possibility of N-isopropylacrylamide (NIPAAm) polymerization under different enzyme actions including cellulose has been demonstrated.[27,28]

In the mild reaction conditions used, the reaction mechanism under cellulase action involves the formation at the surface of a few low molecular weight degradation products with terminal aldehyde groups[29] that tautomerize, leading to the formation of OH glycosidic groups (as was evidenced by ATR-FTIR – band from 1561 cm^{-1}). The reaction between these glycosidic hydroxyl groups in the presence of EDC and NHS took place by bonding of NIPAAm and PNIPAAm onto the fiber surface by tertiary amide bond (band at 1646 cm^{-1} in ATR-FTIR spectra) (Figure 2).

2.4. SEM results

The surface morphology of untreated and modified fibers was analyzed by SEM (Figure 3). One can observe that the surface of untreated fibers was quite homogeneous and that the individual fibers were intact (Figure 3a). When looking closer at untreated CC fibers it was observed that the surface presented ditches. Figure 3b

illustrates the action of modification treatment with NIPAAm, and it can be observed that the fibers show a rougher surface. As evident from the photographs, a thin layer of deposits seems to cover the whole surface.

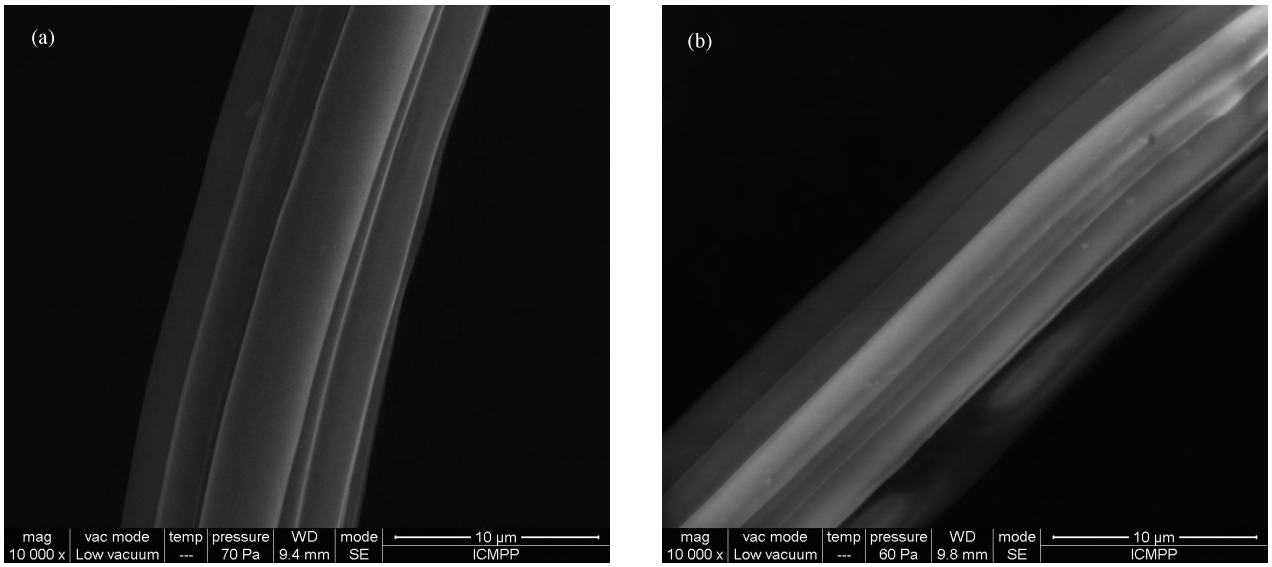

Figure 3. SEM images of the samples (a) CC and (b) CC/cellulase/NIPAAm.

2.5. Swelling degree of the modified fibers

2.5.1. Temperature responsiveness

For temperature responsiveness, swelling was followed in twice distilled water with increasing temperature from 25 to 40 °C. The equilibrium swelling degrees of the fibers modified with NIPAAm obtained by activation with cellulase in comparison with the untreated sample are presented in Figure 4. For the untreated fibers it can be seen that the swelling degree increases with increasing temperature until around 35 °C; at higher temperature the increase in the swelling degree is significantly slower.

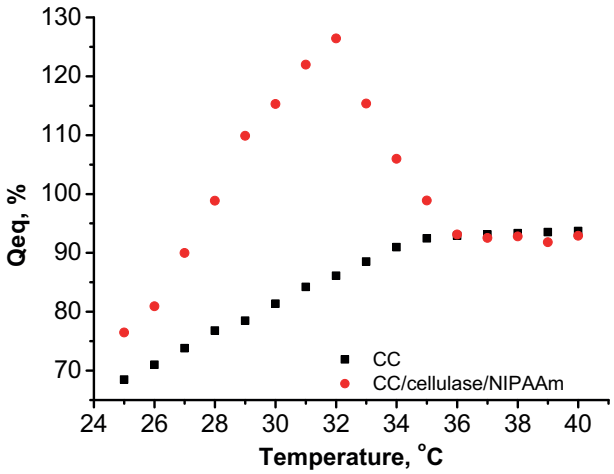

Figure 4. Equilibrium swelling degree as a function of temperature for unmodified CC fibers (■) and CC/NIPAAm obtained by activation with cellulase (●).

The transition temperature was determined with good accuracy by the graphical method. The curves were fitted by a Boltzmann function (using an Origin program) giving the equation:

$$y = \frac{A_1 - A_2}{1 + e^{\frac{(x - x_0)}{dx}}} + A_2, \tag{6}$$

where A_1 is the minimum value of the function; A_2 is the maximum value of the function; x_0 represents the value on the x axis corresponding to the inflection of the curve, equivalent to the transition temperature; and dx is the domain over which this value is found.

For the sample modified with NIPAAm, the variation in the equilibrium swelling degree with temperature is observed, namely the swelling degree increases linearly with increasing temperature until around 32 °C; at higher temperature the fibers suffer a contraction and the swelling degree decreases until around 35–36 °C. The transition is observed around 31.52 °C for CC/NIPAAm. The temperature responsiveness is given only by chains of PNIPAAm; therefore during the treatment a part of NIPAAm polymerized under enzyme action and so it could be also grafted like short chains giving the temperature responsive character, which is revealed by the sudden variation in the swelling degree around 31.52 °C, where a volume transition is found.

2.5.2. pH-responsive character

Chitin is natural, nontoxic, nonallergenic, antimicrobial, and biodegradable. It has a strong positive charge, which allows it to bind with negatively charged surfaces or materials, including metals, skin, and macromolecules such as proteins. Since hydrolysis of chitin by chitinase treatment leads to the release of glucosamine in addition to *N*-acetylglucosamine, it was concluded that glucosamine might be a significant portion of the polymer. Chitin samples have a low amount of 2-amino-2-deoxy-D-glucose units, which can be responsible for the pH sensitivity.[30]

It has been shown that an increase in pH increases the swelling of kraft pulp.[31,32] The very high concentrations of chemicals needed to reach a given pH level can also, due to the high ionic strength in the system, cause a decrease in swelling of the pulp at pH $>$ 10.

Combination of these properties in Chitcel fibers could impart antimicrobial and pH responsiveness to the fibers.[18,19] The introduction of chitin in the blend fiber can improve the water-retention properties of the blend fiber compared to pure fiber.[33]

To emphasize this pH responsiveness the swelling of fibers was achieved in several phosphate buffer solutions of different pH values: 1.2, 3.2, 4.3, 5.5, 7.4, and 9.0, at room temperature. The maximum swelling degree as a function of the different pH solutions that were used for the samples grafted with NIPAAm is presented in Figure 5.

In the case of CC/NIPAAm, swollen in different pH solutions, it is obvious that the maximum swelling degree increases with increasing pH of the solution, from 72.4% in the case of pH 1.2 solution to around 103% in the case of pH 9.0 solution. A sudden variation in the swelling degree around pH 4.50 reveals the pH-responsive character of the fibers.

2.5.3. Swelling kinetics

The swelling kinetics was analyzed for the portion of the water absorption curve with linear variation in the fractional water uptake (M_t / M_{eq}) up to 60% using the following equation:[34]

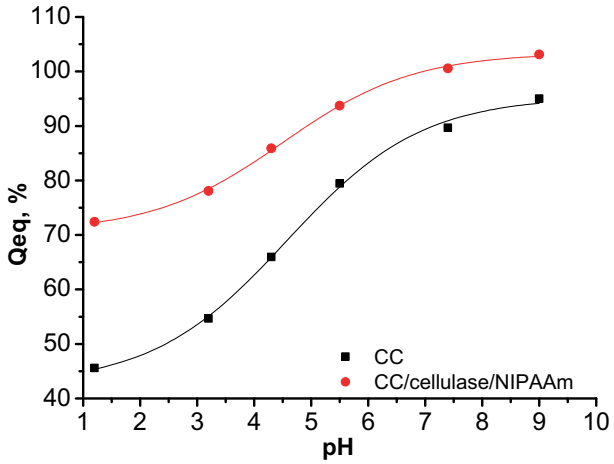

Figure 5. Maximum swelling degree function of different pH solutions used for CC (■) and CC/cellulase/NIPAAm (•).

$$\frac{M_t}{M_{eq}} = kt^n, \tag{7}$$

where M_t is the mass of water absorbed at time t, M_{eq} is the mass of water absorbed at equilibrium, k ($\min^{-n}$) is the characteristic constant of the material describing the rate of swelling, and n is the characteristic exponent describing the mode of the penetrant transport mechanism.

Based on the parameter n, the mechanism of swelling can be classified as follows: when n is equal or less than 0.5, the diffusion mechanism is Fickian, it is case II when n is equal to 1, and for values of n between 0.5 and 1, the mechanism is non-Fickian.[35]

The constant n was calculated from the slopes and intercepts of the plots of $\ln(M_t/M_{eq})$ vs. lnt and the constant k was calculated from the slopes and intercepts of the plots of (M_t/M_{eq}) vs. t^n. The n and k parameters for different pH solutions at different temperatures for all studied samples are presented in Table 7.

Table 7. Parameters n and k for CC/cellulase/NIPAAm in different pH solutions.

pH	CC/cellulase/NIPAAm	
	n	k ($\min^{-n}$)
1.2	0.05	0.78
3.2	0.05	0.78
4.3	0.05	0.79
5.5	0.04	0.83
7.4	0.04	0.83
9.0	0.03	0.86

The values of n for all pH solutions for CC/NIPAAm were between 0.03 and 0.05, which indicated that the transport mechanism was Fickian diffusion.

The constant k slightly increases with increasing pH of the solution for CC/NIPAAm.

It can be observed that n values decrease while rate constant k increases with increasing pH of solution. Since the increase in n means relaxation of the fibers' structure,[36] from Table 7 it is obvious that with increasing pH value the maximum swelling degree also increases and the fibers become more constrained.

In conclusion, cellulose/chitin mix fibers were modified successfully with N-isopropylacrylamide (or PNIPAAm), using cellulase for the fiber surface activation. The modification was confirmed with ATR-FTIR

spectroscopy and X-ray photoelectron spectroscopy. The modification/grafting degree estimated from XPS data was about 47.3%. The morphological changes were evidenced by SEM images. The double responsiveness to temperature and pH was evidenced by the swelling process in twice distilled water with increasing temperature and in different pH solutions at room temperature. It was found that the modified fibers exhibit a LCST of 32 °C and a critical pH around 4.5. This research may be applied to develop intelligent fabrics useful for applications in cosmetic or pharmaceutical fields.

3. Experimental

3.1. Materials

The unwoven CC mix fibers delivered under the trade name of Chitcel by Shandong (China) contain 9–11 wt % chitin, which confers to the fibers an antimicrobial character. Chitcel is a kind of modified cellulose fiber that is produced in a special chemical way by adding the natural antibacterial high molecular weight polymer chitin. The mix fibers possess both the excellent processing properties of viscose fibers and the permanent health functions of antiodor and antibacterial. It is a new kind of viscose fiber concentrating on the advantages of viscose fibers and chitin polymer. Based on the data from elemental analysis, the nitrogen content of the mix fibers is 1.06–1.39 wt%, which corresponds to a chitin content of about 10–13 wt%, close to that provided by the firm's specifications.

Cellulase type Celluclast 1.5 L (a cellulase mixture produced by *Trichoderma reesei*, 6.3 units/mg solid) enzyme, used for the activation of the fibers, was purchased from Sigma-Aldrich. The enzyme was used as received.

N-isopropylacrylamide, used for the impregnation of the fibers, was purchased from Aldrich. The purity was 97% and it was used without further purification.

For the modification of the CC fibers with NIPAAm, two chemical coupling agents were used: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC, molecular weight: 155.24 g/mol, purity: $\geq$97%) as a carboxyl group source incorporated by enzyme activation for the coupling of primary amines to yield amide bonds, and N-hydroxysuccinimide (NHS, molecular weight: 115.09 g/mol, purity: 98%), for increasing coupling efficiency and to create a stable amine-reactive product. Both were purchased from Sigma-Aldrich.

3.2. Modification of the cellulose/chitin mix fibers

Cellulase treatment with Celluclast 1.5 L occurred at 50 °C for 1 h at pH 5 (0.05 M acetate buffer). The fabric-to-liquor ratio was 1:10. After enzyme activation fibers were immersed in solution of N-isopropylacrylamide, 7.5 g/L concentration (solution was made using the same buffer solution as for the enzyme activation), which was previously activated with a mix of the two chemical coupling agents, EDC and NHS, for 4 h, at room temperature under vigorous stirring ($\sim$120 rpm). Then the fibers were centrifuged for 15 min at 800 rpm in order to remove the excess reactant. After modification the fibers were extracted in a Soxhlet extractor with chloroform for 6 h in order to remove the physical absorbed and unreacted chemicals. The modified fibers (CC/NIPAAm) were then dried and analyzed.

3.3. Investigation methods

3.3.1. Elemental analysis

Elemental analysis was used for the determination of the mass fractions of carbon, hydrogen, and nitrogen of the samples. C and H analysis was accomplished by combustion, where a sample was burned in an excess of oxygen at $\sim$700 °C. The Kjeldahl method was used for the quantitative determination of nitrogen.

3.4. ATR-FTIR

The ATR-FTIR spectra were recorded at 4 cm^{-1} resolution with 64 scans by means of a spectrometer, Bruker VERTEX 70, in absorbance mode, by the ATR technique with a 45° ZnSe crystal. Penetration thickness was about 100 μm. For each sample, the evaluations were made on the average spectrum obtained from three recordings. Background and sample spectra were obtained in the 600 to 4000 cm^{-1} wavenumber range. The processing of spectra was achieved using a SPECVIEW program.

3.4.1. X-ray photoelectron spectroscopy (XPS)

XPS measurements were made using a PHI 5000 VersaProbe spectrometer (ULVAC-PHI) equipped with a monochromatic Al Kα X-ray source photon energy = 1486.6 eV. The pressure in the analysis chamber was kept at 2×10^{-6} Pa or lower during each measurement. Measurements were taken at a take-off angle of 45° with respect to the sample surface. Sampling depth was about 10 nm. The MultiPak V8.2C software was used for background subtraction, peak integration, fitting, and quantitative chemical analysis.

3.4.2. SEM analysis

SEM analysis was performed with a scanning electron microscope ESEM—EDAX QUANTA 200, without any further treatments, at 10,000× magnification.

3.4.3. Determination of the swelling degree

Swelling degree represents the quantity of water or solvent absorbed by a substance without dissolution. The swelling properties are controlled by two factors acting in different ways. On one side, the free mixing energy between solvent and the polymeric chains determines the increase in the CC mix fiber volume; on the other side, the elastic response of the macromolecular network opposes the swelling. At equilibrium, the samples reach the maximum swelling degree.[37]

The temperature or pH responsiveness was followed by swelling of the unmodified and modified CC mix fibers in twice distilled water with increasing temperature from 25 to 40 °C in a thermostated bath with a precision of ± 0.2 °C, and/or in several phosphate buffer solutions of different pH, namely 1.2, 3.2, 4.3, 5.5, 7.4, and 9.0.

Swelling studies were performed for all formulations and carried out by direct immersion in the above-mentioned solutions. At predetermined time intervals samples were removed from the solution, gently wiped with a soft tissue to remove excess surface solution, weighed, and then placed back into the vessel as quickly as possible. Swelling degree (Q) was determined by weighing method using the following Eq. (8):

$$Q_{\max} = \frac{(m - m_0)}{m_0} \times 100(\%), \tag{8}$$

where m_0 - weight of dry sample (g) and m - weight of wet sample (g).

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