SYNTHESIS AND CHARACTERIZATION OF N-ALKYL CHITOSAN FOR PAPERMAKING APPLICATIONS

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Chitosan is a natural cationic polymer whose reactive amino groups and primary and secondary hydroxyl groups confer to it interesting properties for papermaking applications, such as cationic polyelectrolyte behavior and high affinity for the cellulose fiber surface. However, the main barrier to these applications of chitosan is the lack of water solubility under neutral pH of the wet-end papermaking system. This paper is aimed at obtaining and characterizing a water-soluble chitosan derivative by introducing alkyl groups along the chitosan chain, to confer to it hydrophobic nature, without affecting significantly its cationic character. The N-alkyl chitosan derivative was obtained by reductive amination, and was investigated by spectroscopic techniques (FT-IR, ^{1}H-NMR, and MS), solubility in water and ionic charge density. The analysis demonstrated the obtaining of alkylated chitosan with a 0.03 substitution degree, completely soluble in water at room temperature, and with a cationic character in a neutral pH medium. These characteristics of N-alkyl chitosan, beside its amphiphilic nature induced by hydrophobic/hydrophilic sequences, make this polymer a very promising additive for papermaking applications.

Keywords: chitosan, N-alkyl chitosan, spectroscopic techniques, acetylation degree, substitution degree, solubility

INTRODUCTION

Chitosan is a polysaccharide, derived from an abundant natural organic resource, chitin, being characterized by a high variability of its physical-chemical properties, due to its natural origin.[1] The term chitosan refers to a heteropolymer chain with β(1→4) linked D-glucosamine or N-acetyl-D-glucosamine residues with different substitution patterns. The unique structural feature of chitosan consists in the presence of primary amines at C-2 position of the D-glucosamine residues and two hydroxyl functionalities.[2-5] These groups allow specific chemical reactions and confer important functional properties to chitosan, which could be further exploited for many applications. Figure 1 illustrates the possible reaction sites of chitosan structural units. Amino functionality provides the potential for chemical reactions, such as acetylation, alkylation, quaternization, grafting and metal chelation. The hydroxyl functional groups also take part at various reactions, such as O-acetylation (*e.g.* O-carboxymethyl, cross-linked O-carboxymethyl chitosans), H-bonding with polar atoms, grafting.[5-8]

Recently, a growing interest has been manifested in the chemical modification of chitosan, for improving its solubility in water, which extends considerably its applications. Derivatization by the introduction of chemical substitutes into the chitosan structure, such as alkyl or carboxymethyl groups, can increase the solubility of chitosan at neutral and alkaline pH values, without significantly affecting its cationic character.[9] Numeorus studies have also been dedicated to the preparation of N-alkyl chitosan derivatives[10-13] with extended antibacterial and therapeutic properties for specific applications in medical industries.[14]

Early researches on chitosan applications in papermaking, performed a few decades ago, are related to its potential function as a

dry and wet strength additive.[15,16] At present, there are only a few applications of chitosan in papermaking, related mainly to surface treatments of special papers. Nevertheless, more and more researchers are now investigating the functionality of chitosan as a papermaking additive for both internal and surface applications.[17-19]

The objective of this research has been to synthesize an N-alkyl chitosan derivative with special features for potential applications in papermaking, under neutral pH conditions. A reductive amination procedure, widely discussed in the literature, was selected for the synthesis of alkylated chitosan derivatives. The method, suggested earlier by Yalpani[20] and modified by Desbrières,[6] involves quantitative grafting of the aliphatic chains on the chitosan backbone. In summary, the procedure develops the equilibrium reaction of an aldehyde with an aminic function of chitosan in acidic solution, to form the chitosan imminium ion, followed by its quantitative reduction with sodium cyanoborohydride (Fig. 2).

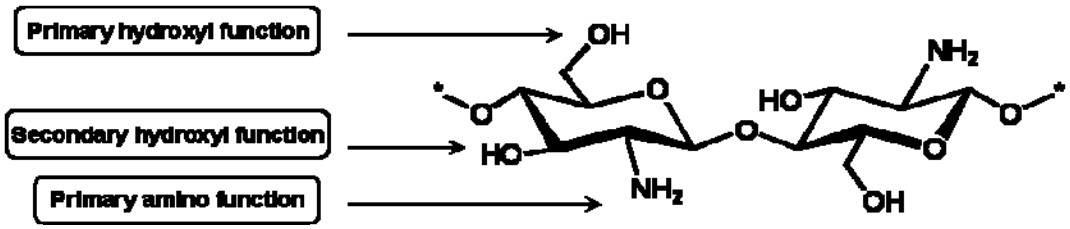

Figure 1: Possible reaction sites for chitosan [5]

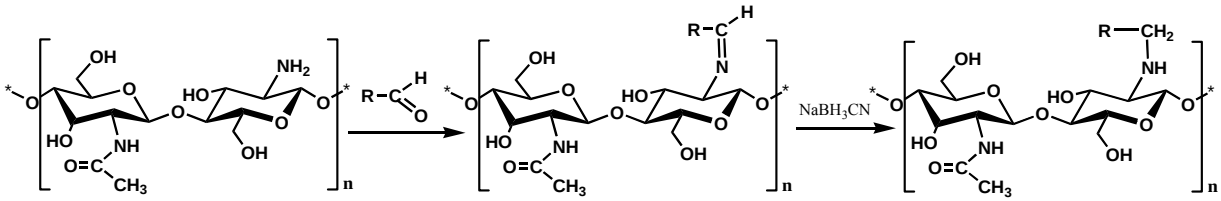

Figure 2: Synthesis of N-alkyl chitosan[21]

EXPERIMENTAL

Materials, methods and analyses

Materials

The following chemicals were used for N-Alkyl chitosan synthesis: chitosan – medium MW (medium molecular weight), deacetylation degree – 90%, cationic charge density – 3.7 meq/g at pH = 4.3 (Vanson, Inc product); octanal – $C_8H_{16}O \geq$ 92% (FCC, Kosher product); sodium cyanoborohydride – reagent grade, 95% (Sigma Aldrich® Inc product); hydrochloric acid – 37% (Sigma Aldrich Co. product); sodium hydroxide; ethanol; methanol and 0.001 N PVSN as standard polymer for colloidal titration (Mütek Co supply).

Analysis methods

Three spectroscopic techniques were used to assess the success of the synthesis reaction: FT-IR spectroscopy – Digilab FTS 2000 Fourier transform spectrometer, domain 4000-400 cm^{-1}, resolution – 4 cm^{-1}, 32 scans; the FTIR spectra were processed with the KnowItAll® Informatics System program and then normalized; ^{1}H-NMR spectroscopy – Bruker Avance DRX 400 spectrometer, 16 scans, using D_2O as a solvent; Mass spectroscopy – Mass selective detector (MSD) 5975 inert XL Agilent Technologies. Colloidal titration was performed on a Mütek PCD-02 apparatus, to measure the charge density of polymers.

Synthesis of N-alkyl chitosan derivative

The primary amino groups of chitosan undergo a Schiff reaction with aldehydes and ketones, to yield the corresponding aldimines and ketimines, which are converted to an N-alkyl derivative by reduction with sodium borohydride ($NaBH_4$) or sodium cyanoborohydride ($NaBH_3CN$), among other reducing agents.[1] In the present study, N-alkyl chitosan was prepared in several steps, presented in Figure 3, and namely, dissolution of chitosan in 0.1 M acetic acid solution; addition of octanal ($C_8H_{16}O$) and reducing agent ($NaBH_3CN$) under continuous stirring, at room temperature; adjusting of pH to 8-10 with a NaOH solution, after a 20-24 h

reaction time; separation of the precipitate by centrifugation; precipitate washing with ethanol/water mixtures (50/50 and 70/30 volume ratios) and finally only with ethanol; precipitate drying at 50 °C, under air circulation; dissolution of N-alkyl chitosan in a 0.1 M HCl solution, to obtain its salt (water-soluble derivative); precipitation of N-alkyl chitosan hydrochloride by adding methanol (2:1 v/v) and then HCl (37%), corresponding to 1.02 mol/L HCl, to the final volume; centrifugation and drying of the final compound. The overall yield of N-alkyl chitosan synthesis was between 88-91% for three duplicate syntheses.

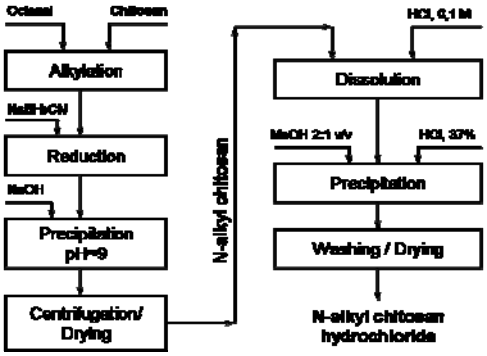

Figure 3: Synthesis scheme of N-alkyl chitosan (hydrochloride salt form)

RESULTS AND DISCUSSION

FT-IR analysis

Infrared spectroscopy is one of the most important and widely used analytical techniques available to scientists working on chitosan. The growing interest in the chemical modification of chitosan to improve its solubility and applications established that the most important application of infrared spectroscopy in this respect is the structural analysis of the chemically modified forms of chitosan.[22-24]

Figure 4 presents the spectra of N-alkyl chitosan and of original chitosan. The FTIR spectrum of chitosan shows peaks assigned to the polysaccharide structure at 898 cm^{-1} and 1154 cm^{-1} and a strong amino characteristic peak at around 1575 cm^{-1}. The absorption bands at 1650 cm^{-1} and 1320 cm^{-1} are characteristic of N-acetylated chitosan, and have been reported to be the amide I and III bands, respectively. Obvious changes of the FTIR spectrum are observed after chitosan alkylation, suggesting that substitution has occurred on the amino groups of chitosan. Peak intensity at 1575 cm^{-1} decreases, due to conversion of $-NH_2$ to N-alkyl substituent, a new peak appearing at 1519 cm^{-1} – corresponding to asymmetrical stretching of C–H in the methyl groups.

Substitution degree (DS) of N-alkyl chitosan

As the FTIR spectra evidence that alkyl-substitution occurs on the amino groups, the substitution degree (DS – number of chains grafted per glycoside unit of chitosan)[25] could be evaluated by the decrease of the deacetylation degree (DDA) of N-alkyl chitosan.

Various analytical techniques have been developed to measure the DDA of chitosan, among which infrared spectroscopy has received special attention.[23] In the present study, the DDA (average number of D-glucosamine units per 100 monomers, expressed as percent value) of both original and N-alkylated chitosan was calculated by applying data of IR spectra in relation (1):[26]

$A_{1320}/A_{1420} = 0.3822 + 0.03133(100 - DDA)$

The following results were obtained:

Original chitosan, $DDA_1 = 86.05\%$
N-alkylated chitosan, $DDA_2 = 82.32\%$
N-alkyl chitosan, $DS = (DDA_1-DDA_2)/100$
$\rightarrow DS = 0.037$.

^{1}H-NMR analysis

The ^{1}H-NMR spectra of chitosan and N-alkyl chitosan in D_2O, obtained by a Bruker Avance DRX 400 spectrometer, at a resonance frequency of 400 MHz, are shown in Figure 5. The ^{1}H-NMR spectra were performed at a temperature of 300 °K. The ^{1}H-NMR spectrum of chitosan presents a signal between 3.10 and 2.90 ppm, corresponding to the hydrogen bonded to the carbon atom C_2 of the glucosamine ring, while the signals between 3.30 and 4.00 ppm correspond to the hydrogen atoms bonded to

carbons C_3, C_4, C_5 and C_6 of the glucopyranose units.

The ^{1}H-NMR spectrum of modified chitosan displays broadening of the characteristic peaks and signals in the 1.7-0.9 ppm region, attributed to the protons of the methyl (-CH_3) and methylene (-CH_2-) groups grafted onto the chitosan chain, which evidences the chemical modifications resulting from the alkylation reaction. The broad multiplet peaks from 1.3 to 1.7 ppm are attributed to the methylene hydrogens of the -CH_2- groups, while a typical peak at 0.9 ppm corresponds to the methyl protons at the terminal groups -CH_3, both belonging to the -C_8H_{17} aliphatic chain.[6,27,28,29]

Substitution degree (DS) of N-alkyl chitosan

The ^{1}H-NMR spectra provide information that can be used to evaluate the acetylation degree (DA) of the original chitosan and the substitution degree (DS) of chitosan derivatives.[6,22,30] From this spectral analysis, and taking into account the intensity of the signals due to the hydrogen bond to C_2, the hydrogen atoms of the acetamide groups of chitosan and the protons of grafted alkyl chain, the DA and DS values were calculated[30,31] by applying relations (2) and (3):

$$DA = \frac{\frac{I_{CH_3}}{3}}{I_{H-1} + I_{H'-1}} \quad (2)$$

$$DS = \frac{\frac{I_{(CH_2)_7}}{14}}{I_{H-1} + I_{H'-1}} \quad (3)$$

where I_{H-1}; $I_{H'-1}$ I_{CH3} and $I_{(CH2)7}$ represent the integrals of the signals of H-1 (the anomeric protons of the D-glucosamine units), H'-1 (the anomeric protons of the N-acetyl-D-glucosamine units), -CH_3 protons and protons of the grafted alkyl chain, respectively.

Based on relation 2, the acetylation degree of original chitosan (DA) was of 11.75%, corresponding to a deacetylation degree (DDA) of 88.25% (DDA = 100-DA). Comparatively with the DDA calculated from FT-IR spectra (86.05%), the ^{1}H-NMR value is fitting tightly to that provided by the chitosan supplier (DDA 89%). This finding is consistent with the authors who state that NMR spectroscopy yields the most reliable results in the determination of DA and DS values.[31]

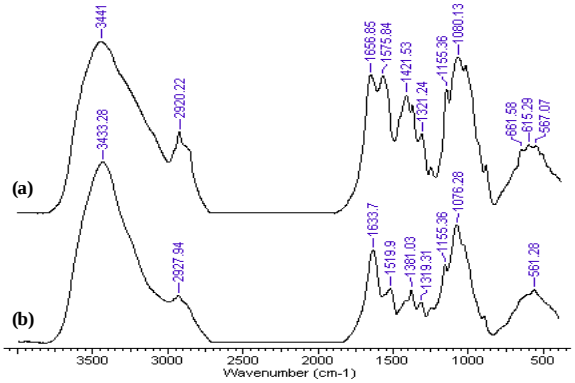

Figure 4: FT-IR spectra of chitosan (a) and N-alkylated chitosan (b)

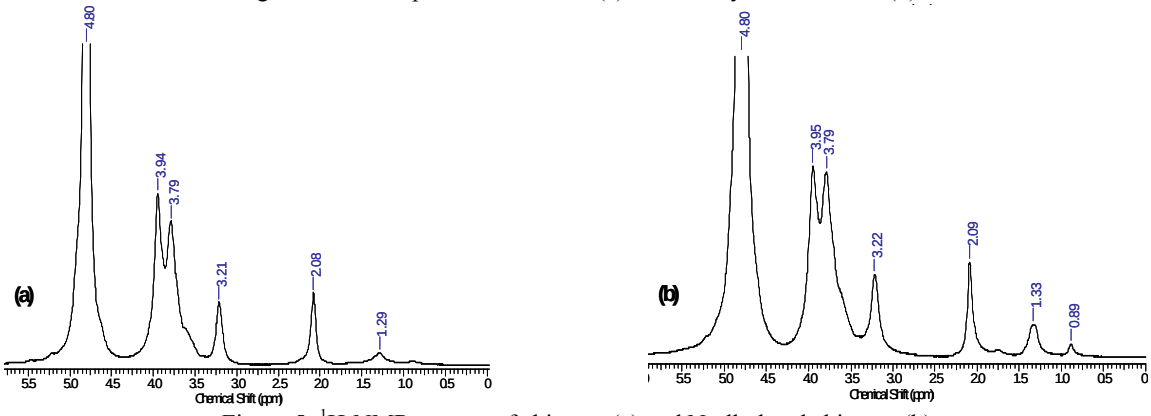

Figure 5: ^{1}H-NMR spectra of chitosan (a) and N-alkylated chitosan (b)

The value of the substitution degree calculated by relation (3) is 0.03, a little lower than that obtained from FT-IR spectra (0.037). Based on the glucose-amine unit/aldehyde ratio used in the synthesis of N-alkylated chitosan, the theoretical DS is 0.07, evidencing that, under the selected conditions (glucose-amine unit/aldehyde ratio, temperature, alkyl-chain), the yield of the substitution reaction is around 45%.

Mass spectroscopy

Mass spectroscopy (MS) is a destructive analytical technique used for measuring the characteristics of individual molecules.[32] Molecular mass, molecular structure and sample purity are important data that can be obtained from mass spectrometry analysis.[23,33,34] The molecular weight of the studied samples was read on the mass spectra presented in Figures 6 and 7. Molecular weight represents the heaviest ion fragmented from the sample, resulting by the loss of an electron from the molecule. In the case of chitosan (Fig. 6), the molecular ion is seen as a small peak at m/z = 2323 while, for N-alkyl chitosan (Fig. 7) – at m/z = 1678.

The qualitative and quantitative data provided by mass spectroscopy could be used for a possible structure of a compound by observing its fragmentation. In this study, the analysis of the mass spectrum allowed schemes of sequential fragmentation of the glycoside bonds and rings for chitosan and N-alkyl chitosan, as presented in Figures 8 and 9. Figure 8 shows that fragmentation of a chitosan molecule occurs through sequential cleavage of glycoside linkages, the most abundant fragments corresponding to the C_4-O- bond cleavage at charge ratios (m/z) of 162, 323, 484, 645, 806, 967, 1128, 1289, and so on. In the case of the N-alkyl chitosan molecule, besides the C_4-O- bond cleavage characteristic of chitosan, other fragmentations occur at the following charge ratios: 274 and 290, 435 and 451, 596 and 612, 757 and 773, 918 and 934, 1079 and 1095, 1240 and 1256. These fragments are characteristic of the alkyl chains grafted to the glucosamine units, confirming the chemical modification of chitosan.

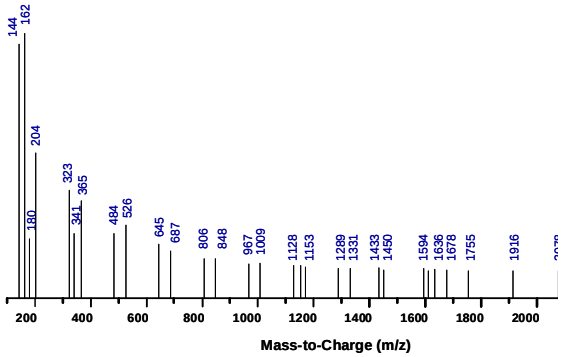

Figure 6: Mass spectrum for chitosan

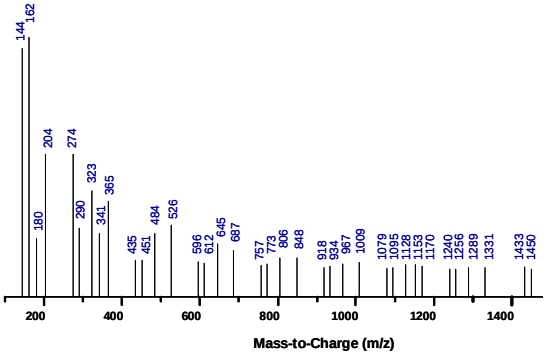

Figure 7: Mass spectrum for N-alkyl chitosan

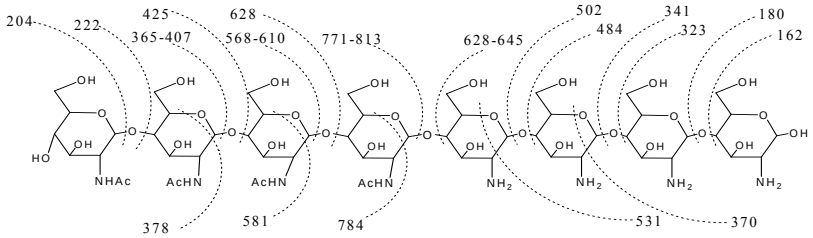

Figure 8: Possible fragmentations of the chitosan molecule

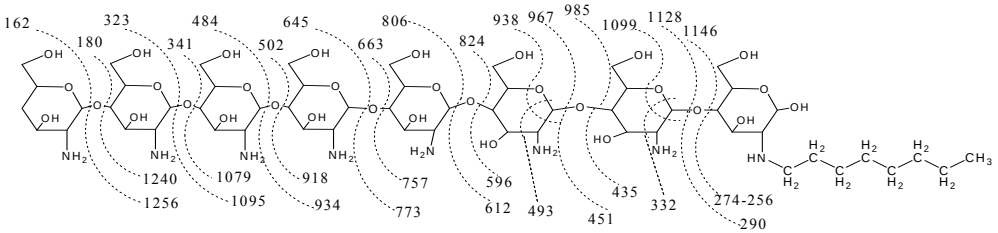

Figure 9: Possible fragmentations of the N-alkyl chitosan molecule

Table 1
N-alkyl chitosan solubility at different concentrations

Concentration, g/L	Solubility, %
1	100
5	100
10	99.4

Table 2
Charge density of polymers at two pH levels

pH	Cationic charge density, meq/g	
	Chitosan	N-alkyl chitosan
4.0	3.70	3.73
7.0	1.18	1.26

Solubility test

To estimate the solubility of N-alkyl chitosan, the chitosan derivative was mixed with water to obtain different concentrations (1 g/L, 5 g/L and 10 g/L), stirred for 3 h at room temperature and then filtered through a 0.45 μm filter paper. Solubility was estimated from the change in filter paper weight and was calculated as percent of soluble chitosan related to the total mass of chitosan.[35] The water solubility values of chitosan derivatives are listed in Table 1. This level of solubility is adequate for the current conditions of additive dosing in wet-end papermaking systems.

Colloidal titration

Colloidal titration was performed on a Mütek PCD-02 apparatus (streaming current detector), to measure the ionic charge density of the original chitosan and N-alkyl chitosan, respectively. This method is based on the measurement of the streaming current produced by the charged polymer molecules adsorbed on a moving surface. Solutions (1 g/L) of the two polymers have been prepared, in water for alkylated chitosan, and in 0.1 M acetic acid solution for the original chitosan. To calculate the ionic charge at two different pH values, the samples were prepared by diluting 1 mL solution (1 g/L – chitosan or alkylated chitosan) to 10 mL volume with buffer solutions (pH = 4.0 and 7.0). Direct colloidal titration was performed in the measuring cell of the Mütek PCD-02 apparatus, with the standard polymer PVS-Na 0.001 N. The values of charge density for the two polymers are given in Table 2.

As expected, at such low substitution degree (DS = 0.03), the cationic charge of N-alkyl chitosan does not differ essentially from that of chitosan. On the contrary, the cationic charge of alkylated chitosan increases at both pH levels. Obviously, one cannot draw conclusions on this aspect, and it would be interesting to analyze what happens at higher substitution degrees.

CONCLUSIONS

N-alkyl chitosan with a low substitution degree (DS = 0.03) was successfully synthesized by a Schiff reaction. The structure of chitosan derivatives was confirmed by three different spectroscopic techniques, namely, FT-IR, ^{1}H-NMR and MS:

- the FT-IR spectra evidence the chemical modification of chitosan, namely, the disappearance of the peak at 1575 cm^{-1}, due to the conversion of -NH_2 into an N-alkyl substituent, and the occurrence of new bands at 1519 cm^{-1}, corresponding to C-H stretching into methyl groups;
- the ^{1}H-NMR spectra of N-alkyl chitosan display characteristic peaks in the 1.7-0.9 ppm region, attributed to the protons of the methyl (-CH_3) and methylene (-CH_2-) groups grafted onto the chitosan chain, which also support the chemical modification of chitosan;
- the mass spectra of N-alkyl chitosan present several peaks, characteristic of the alkyl chains grafted onto glucosamine units, confirming again the N-alkylation of chitosan.

Both the FT-IR and ^{1}H-NMR spectra provide reliable information for calculating the acetylation/deacetylation degree of chitosan, as well as the substitution degree of alkylated chitosan. However, the observation was made that the deacetylation degree of chitosan calculated from the ^{1}H-NMR

spectra is tightly fitted to that provided by the chitosan supplier, this conclusion being consistent with those of the authors who state that NMR spectroscopy yields the most reliable results in the determination of DA and DS values.

The hydrochloride salt of the synthesized N-alkyl chitosan is completely soluble in water at room temperature, up to 1% concentration. The derivative displays a cationic charge close to that of chitosan, in both acid and neutral pH media.

These characteristics of N-alkyl chitosan, besides its amphiphilic nature, induced by hydrophobic/hydrophilic sequences, could be exploited for its applications as a multifunctional additive in papermaking, for hydrophobization of cellulose fibers and paper surfaces, simultaneously with dry strength improvement; improvement and control of retention and drainage of the paper stock; adsorption of the sticky contaminants from process water. In this respect, further investigations are necessary to optimize the targeted properties of N-alkyl chitosan: substitution degree, alkyl chain length and electro-kinetic behavior.

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