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Article

Grafting of Chitosan with Fatty Acyl Derivatives

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A plastificação interna da quitosana pela inserção covalente de longas ramificações alifáticas, tipicamente 12C, foi conduzida por meio da reação de condensação entre os grupamentos amino da quitosana com os derivados ácidos do ácido láurico, como anidrido ou cloreto de lauroíla que são mais reativos do que o ácido correspondente. A rota química possibilitou a *N*-acilação seletivamente. O grau de substituição, determinado quatitativamente por FTIR e ^{1}H NMR, variou entre 3 e 35%. A análise quantitativa por FTIR baseou-se em um método de calibração que forneceu boa precisão nos resultados. A quitosana modificada mostrou-se solúvel em água neutra e/ou DMF conforme o grau de substituição.

The internal plasticization of chitosan with covalently linked long aliphatic branches, typically 12C, was accomplished through the condensation of the amino groups of chitosan with acidic derivatives of lauric acid, as lauroyl anhydride or lauroyl chloride, that are more reactive than the fatty acid itself. The chemical pathway led to selective *N*-acylation. The degree of substitution was quantitatively determined by FTIR and ^{1}H NMR and varied between 3 and 35%. The FTIR quantitative analysis was based in a calibration mmethod with good accuracy. The modified chitosan products were soluble in neutral water and/or DMF according to the degree of substitution. The modified chitosan films were more flexible than the pristine, non-modified ones.

Keywords: chitosan, grafting, lauroyl chitosan, fatty acyl derivatives

Introduction

Chitosan is the usual name for the polymer made up of β-(1→4) 2-deoxy 2-amine D-glucose units (Figure 1a), which is obtained from the total or partial deacetylation of chitin which in turn is made up by 2-acetoamide-2-deoxy-D-glucose residues linked through β-(1→4) bonds (Figure 1b). Usually the term chitosan is employed when chitin is deacetylated to about 50%. Thus, chitosan is the *N* deacetylated derivative of chitin, although the *N*-deacetylation is almost never complete. Both structures are similar to cellulose (Figure 1c). After cellulose, chitin is the most abundant natural polymer, and is found mainly in the exoskeleton of crustaceans, mollusks, insects, etc. rendering the material very interesting for a variety of applications.[1] Chitosan is a functional biopolymer biomedical industrial potential.[2-10] Due to its biocompatibility and biodegradability[2] combined with non-toxic, non-antigenic and antimicrobial properties the material is potentially useful in several biomedical applications such as tissue regeneration, controlled drug release, cell immobilizing gel systems.[11] Many investigations have been carried out on chitosan modification to obtain polysaccharide-based advanced materials with unique bioactive properties.[12-17] These upgrading include, among others, grafting with oligo ethyleneglycol methacrylate,[18] with flavonoids extracts,[19] with alyl and sulfate groups and solubilization of anticancer drugs into the polymer micelles by physical entrapment,[20] and chloroacetylation.[21]

The major shortcoming in the practical usage of chitin and chitosan is the rigidity of their chains, resulting in poor solubility and difficult processing. Chitin is soluble only in strong solvents as *N,N*-dimethylformamide (DMF), usually with the aid of compounds capable of breaking hydrogen bonds, such as lithium chloride,[22] whereas chitosan is soluble in acidic aqueous media. The molding of both polymers by usual techniques such as injection or extrusion is hindered by their high thermal stability. In spite of the large number of publications on chitosan modification, a systematic study of the internal plasticization with the grafting of alkyl chains is still lacking.

In this contribution we report a means of improving chitosan processibility through the controlled insertion

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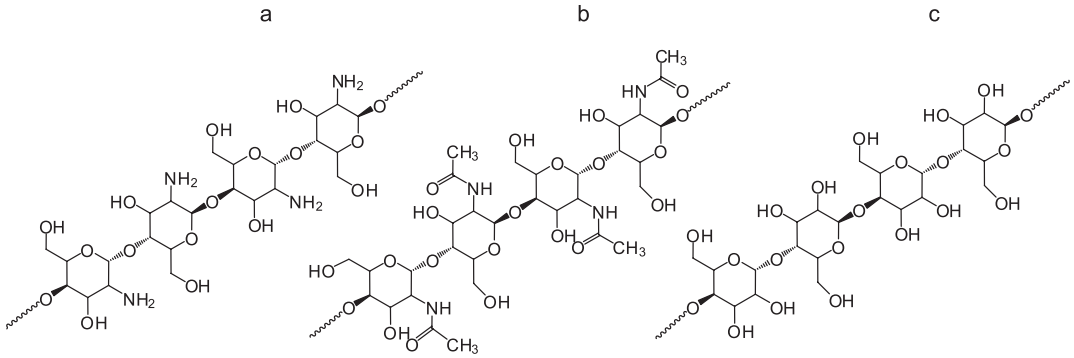


Figure 1. Structures of (a) chitosan, (b) chitin, (c) cellulose.

of covalently linked long aliphatic chains, which in a systematic way acted like as internal plasticizer.[22,23] The free volume introduced by the flexible grafted chains was responsible by the plasticization effect, separating the chains and at the same time breaking the hydrogen bonds that account for the high cohesion of the original polymer. The correlations between the degree of acetylation, the physical and biomedical properties is an ongoing project of our laboratory.

Lauroyl (12C) branches were inserted in concentrations varying from 3 to 35%, (mol 12C/100 mol glucose ring in relation to the original chitosan). The chemical strategy (Figure 2) was based in the reaction of the amino and hydroxyl groups of the polymer with the acid groups of lauroyl anhydride or lauroyl chloride. The choice of 12C insertion was based in the availability of lauric acid derivatives and the absence of other chemical groups in the chain as hydroxyl or double bonds, such as in 16C (palmitoleic, one double bond), 18C (oleic, one double bond), 18C ricinoleic (one double bond and one hydroxyl group) acid derivatives, that could interfere in the analysis of the final grafted chitosan chains.

This approach has been followed before but not in a systematic way and generally aimed to use the acetylated product for further reactions such as esterification and adsorption of heavy metals,[24] or to study the rheological,[25] interfacial and electrical,[26] properties of the substituted materials.[18-29]

The analytical method employed for the quantitative determination of the degree of insertion (DI) of ramifications in the resultant grafted polymer, was based on FTIR band correlation. A similar procedure has been used by other authors,[25,30] but only aiming to quantify the degree of acetylation of chitosan, which is the acetyl content that remained in chitin after deacetylation. Grafted chitosan is usually analyzed by means ^{1}H or ^{13}C NMR, but the shortcoming of the last method is that solubility depends on the DI. The backbone has a hydrophilic nature, whereas the grafted chains are hydrophobic, thus the balance between main chain/ramifications will dictate the solubility behavior. Consequently each DI range presents a different solubility character and NMR analysis will be limited by the availability of the appropriate deuterated solvent for the particular DI under test.

Experimental

Materials

Reagents

Lauroyl anhydride (PA, Across) thionyl chloride, Cl_2SO (PA, Reagen), KBr (spectroscopic grade, Vetec), KOH

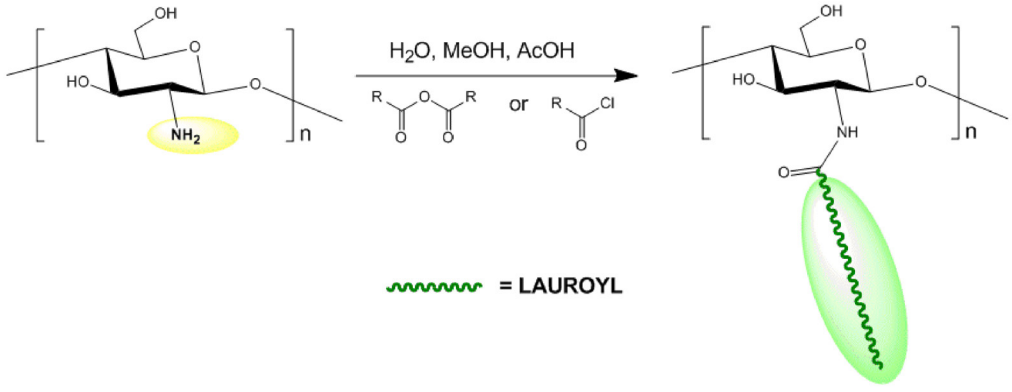


Figure 2. Chemical route to the insertion of lauroyl side groups onto the chitosan backbone.

(PA, Vetec) dehydrated calcium chloride (PA, Vetec) and lauric acid (USP grade, Henkel) were used without further treatment. Pyridine was dried with KOH and distilled at normal pressure (T = 112 °C) and stored over 4 Å molecular sieves. Chitosan in powder form was kindly supplied by Polymar Brazil, with maximum degree of acetylation of 15% and Mn *ca.* 2.9×10^5 was used without any further treatment.

Solvents

Acetone, methanol, ethanol, *N,N* dimethylformamide (DMF), *N,N*-dimethylacetamide (DMAc), *N*-methylpyrrolidone (NMP), *n*-hexane, ethyl ether and acetic acid, were PA grade and used without further purification. Distilled and deionized water was used with a resistivity > 18 MΩ.

Equipment

FTIR spectra were acquired with a Biorad spectrometer, KBr pellets, using a scan range of 4000 to 400 cm^{-1}, 32 scans, and resolution of 4 cm^{-1}. The analyses were performed at room temperature (20-25 °C) and under a controlled relative humidity of 40 up to 60%.

^1H NMR spectra were recorded with a 200 MHz Brücker spectrometer, using deutered water or deutered dimethylformamide as solvent and TMS as internal standard.

Dynamical mechanical thermal analysis tests were performed with the DMTA equipment Netzsch DMA 242 in the tensile mode, in the temperature range –100 to 300 °C, 50 Hz, under N_2 at 50 mL min^{-1}, heating rate 3 °C min^{-1}. The results were interpreted as reported elsewhere.[31]

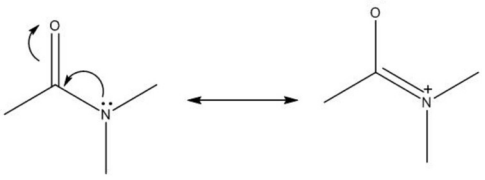
Procedures

Lauroyl chloride was prepared by the reaction of lauric acid with an excess of thionyl chloride in *n*-hexane under reflux during one hour. The product was distilled under reduced pressure. The purity was 95% as determined by titration of a hydrolyzed aliquot with 0.1 mol L^{-1} NaOH and further confirmed by gas chromatography.

Chitosan was converted to lauroyl chitosan with lauroyl chloride in methanol, at low temperature and constant stirring, with the former dissolved in dry pyridine. Typically, a 2% solution of chitosan in water/methanol/acetic acid was diluted with an excess methanol and cooled to –5 °C with constant stirring. A predetermined amount of lauroyl chloride dissolved in dry pyridine was slowly added under vigorous stirring. The reaction proceeded overnight and the final product was then filtered, washed with ethanol/acetone, re-precipitated twice and after dissolution was poured in a Petri dish, de-aerated and slowly dried in air. The formed film was dried under vacuum at 35 °C during 48 h.

Results and Discussion

Chitosan is a multi-nucleophilic polymer due to the presence of the *N*-amino and hydroxyl functional groups. The initial sites where substitution occurs are the more nucleophilic *N*-amino groups which are readily protonated in acid medium. *N*-acylation involves a reaction between the polymer and an acid anhydride or acyl halide which proceeds through an addition/elimination type mechanism, where amide functionality of the *N*-amino groups is restored as in the chitin precursor. These reactions are driven toward amide formation because amides are more stable molecules (compared to acyl carbonyls) as explicable in terms of resonance localization of the lone pair electrons on nitrogen into the carbonyl p system which can be depicted as reported elsewhere.[32,33]



The degree of lauroyl groups inserted was systematically varied through the variation of the amount of lauroyl chloride added to the reaction. Since the *N*-acylation reaction is favored, we have used a diluted solution in methanol, at low temperatures, thus favoring the former reaction, minimizing the occurrence of *O*-acylation in carbons 3 and 6 of the chitosan main chain. Materials with degree of insertion as low as 3% were soluble in slightly acidic or neutral water, whereas those with medium (10%) or higher (more than 40%) content of grafted chains were soluble in methanol and dimethylformamide respectively.

FTIR characterization

To quantify the degree of insertion (DI), an analytical model was developed based on the infrared absorption of the starting materials and final product.

The best results obtained studying the FTIR method to quantify the degree of insertion were based on the height ratios of the CH and CO bands of at 2926 and 1087 cm^{-1}, depicted in Table 1.

A calibration curve was built up with blends with different ratios of lauric acid and chitosan. Since the location and shape of the absorptions relative to the branches and to the glycoside ring do not change in lauryl chitosan as compared to the starting materials, the ratio between the areas or heights of the CH stretching (relative to the aliphatic branch) and of the CO bending (relative

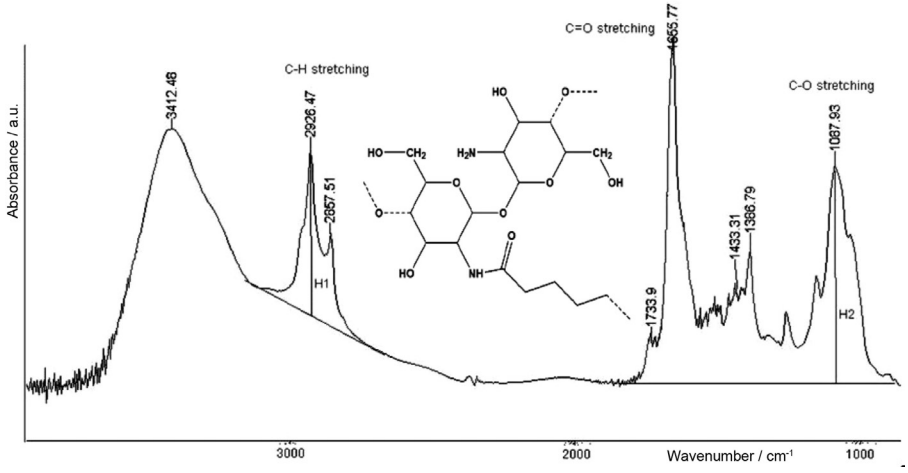


Figure 3. FTIR spectrum of grafted chitosan sample with DI = 35%.

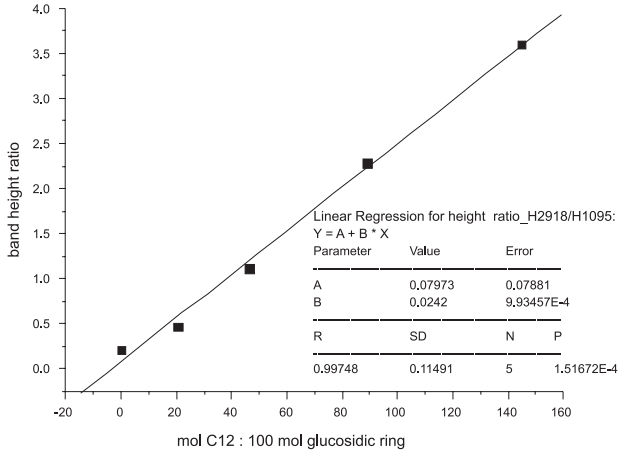


Figure 4. Calibration curve based on data Table 2 for the quantitative determination of the degree of insertion. Linear fit of height ratio 2926/1087 cm^{-1}.

to the glycoside ring) bands was plotted against the lauric acid/chitosan blend composition. This way the errors introduced by differences in optical path due to thickness variations in the KBr pellet were suppressed. This approach gives a fast and convenient procedure to quantify DI. The best resolved bands were 2926 cm^{-1} (CH stretching) and the one located at 1087 cm^{-1} (CO bending), as shown in the spectrum of Figure 3. The calibration curve shown in Figure 4 was plotted using data displayed in Table 2. It was observed that the *O*-acylation was practically eliminated, since the ester band, typically above 1700 cm^{-1}, was not seen in the spectra of almost all samples. When present, as in the spectrum of Figure 3, its height was minimal in comparison to the amide carbonyl at 1665 cm^{-1}.

NMR characterization

Most spectra were acquired using neutral deuterated water solutions. For low graft degrees a slight decrease in pH was needed, and for those with higher graft degrees, the spectra were run in deutered DMF. The assignment of the aliphatic peaks in lauroyl chitosan was made by comparing with the spectra of the starting chitosan (Figure 5). The signal of the aliphatic hydrogen atoms was located around 3 ppm[34] and was attributed to two hydrogen absorptions: one at the ring and two neighbors to the carbonyl group. These two absorptions were enough for the determination of the DI, a complete assignment of the NMR spectra is much

Table 1. Correlation indices obtained with several absorption pairs of FTIR absorption bands for the quantification of the degree of insertion (DI) of lauroyl groups onto chitosan backbone.(A) stands for band area and (H) for band height

Band ratio	Correlation index
A1470/A1095	0.89
A1470/A1074	0.91
A2918/A1074	0.97
A2918/A1095	0.96
H1470/H1074	0.98
H1470/H1095	0.97
H2918/H1074	0.995
H2926/H1087	0.997

Table 2. DI results using height ratio between bands at 2926 cm^{-1}(C12 branch) / 1087 cm^{-1} (glycoside ring); (a) DI= Inserted lauroyl groups / 100 glycosides units, molar ratio; (b) band height ratio

DI (a)	H2926 / H1087 (b)
0	0.21074
21	0.46961
46	1.11515
89	2.28523
145	3.59567

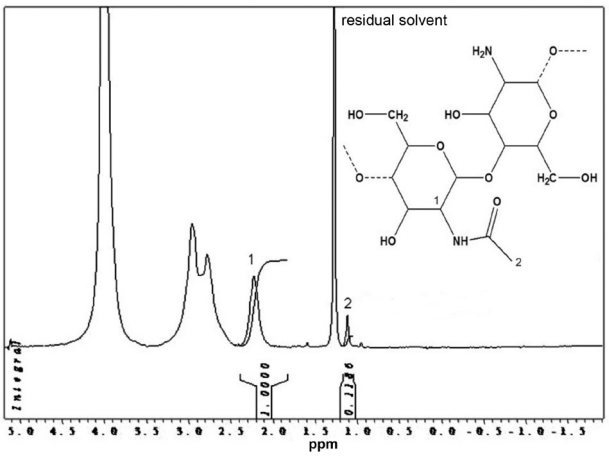


Figure 5. ^{1}H NMR spectrum of pristine chitosan.

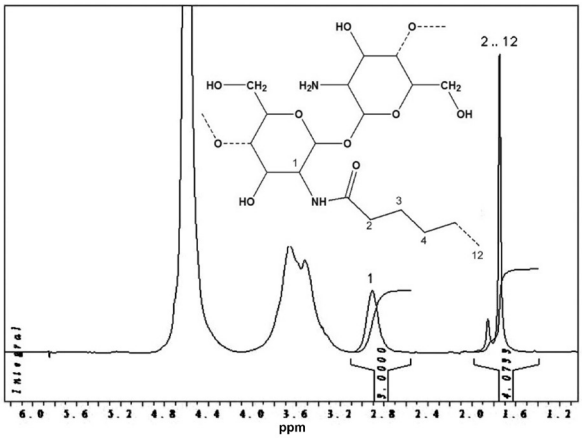


Figure 6. ^{1}H NMR spectrum of chitosan containing lauroyl side groups.

more involved and unnecessary. A typical NMR spectrum of lauroyl chitosan is depicted in Figure 6. It is noteworthy to mention that the initial DI of the starting polymer (maximum 15%), due to residual acetoyl branches has little effect either in the absorption in the aliphatic region and in the plasticization effect, as compared to the C12 branches.

The comparison between the analytical methods employed afforded a correlation index of 0.9989 as shown in Table 3.

The strategy here described was successful in preparing flexible chitosan films. The plasticization is permanent since the elements incorporated (the long aliphatic chains) are covalently bonded to the polymer backbone. Figure 7 illustrates the relative size of the inserted chains as compared to the chitosan backbone, in a computer optimized structure of 6 units of pristine chitosan (a) and one lauroyl chitosan branch (b).

Preliminary tests to evaluate the effect the insertion of long branches on thermal and mechanical properties of chitosan were run using differential scanning calorimetry (DSC) and dynamical mechanical thermal

Table 3. Comparison of FTIR and ^{1}H RMN results for the quantitative determination of inserted lauroyl branches in chitosan backbone, mol%/100 mol (mol of inserted branches *per* mol of glucoside rings)

Sample	DI (FT-IR)	DI (^{1}H RMN)
QNH2	0	0
RC034	3.0	3.2
RC044	7.5	7.7
RC042	10.1	10.6
RC043	12.3	11.4
RC037	15.1	15.6
RC045	34.6	35.3

Correlation index (R^2) = 0.9989.

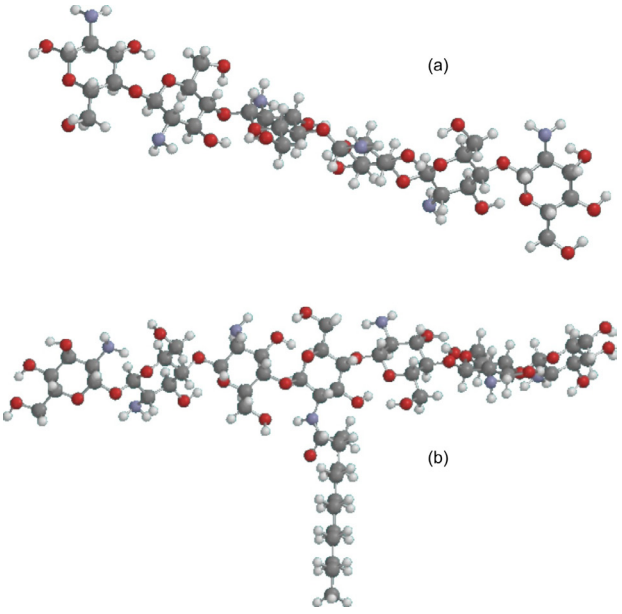


Figure 7. Computer optimized structure of 6 units of pristine chitosan (a) and of lauroyl chitosan (b) (online version in color).

analysis (DMTA). The DSC data did not afford conclusive results; the variation of Cp with temperature was too subtle and not reliable. The DMTA technique was tried, and provided more straightforward data. However, it is not free of problems, though. The literature reports a series of relaxations, around the peak assigned to the glass transition, which are accounted to water molecules, linked to the main chain in a fair large ways: adsorbed, weakly and/or strongly bonded, hydrogen bonds and so on.[35,36] Based on these reported data, we have assigned the transitions presented in Figure 8. The glass transition of the pristine polymer was detected at 147 °C and that of the modified chitosan, appeared as a shoulder in the region 50 to 105 °C. These are in fair agreement with reported data for chitosan and chitosan derivatives. It is noteworthy that chitosan is a natural product, derived from the deacetylation of chitin,

which is never one hundred percent complete. Therefore, the final properties must reflect these variations, apart from the modifications introduced.

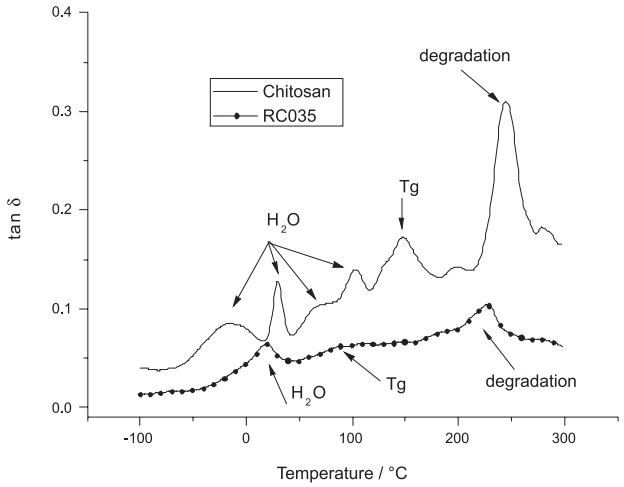


Figure 8. tan δ of pristine (solid line) and of substituted chitosan (dotted line) with 44% of degree of insertion.

Taking into account that the grafted chains are built up by a fatty acid derivative, it is expected that the biocompatibility and biodegradability of the original was preserved, since chitosan and fatty acid derivatives are both completely biodegradable and in the majority of cases, biocompatible as well. Moreover, reported results have shown that the compatibility increases with the length of the side branch.[37] The pristine dry chitosan films were brittle materials, which could not support folding without breaking, whereas those with a DI of 5% could be bent and folded, and those with a higher DI supported twisting. The quantitative correlations of the mechanical properties with DI and corresponding morphology is now under way, along with the influence of the size of the grafted chain.

Conclusions

The preparation of lauroyl chitosan with degrees of inserted ramifications changing from 3% up to 35% (m/m) was successfully achieved. The reaction was run in conditions leading to essentially complete *N*-acylation, minimizing substitution at the hydroxyl groups in C3 and C6 glucoside units. The quantitative characterization was based on the chemical dissimilarity between the chitosan backbone and inserted aliphatic groups allowing the use of a FTIR band correlation method which validity was further corroborated by NMR spectroscopy. As the method is useful for the preparation of chitosan derivatives with varying degree of branching, the correlation between the structure and mechanical/thermal properties could be addressed.

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