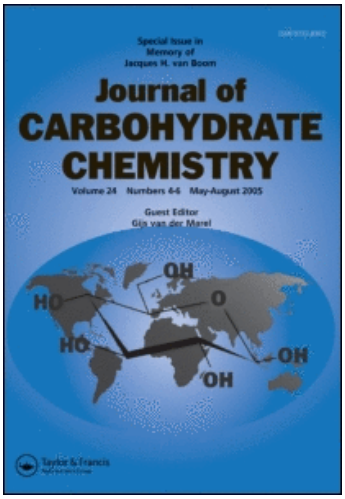

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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To cite this Article Rodrigues, Máira R.(2005) 'Synthesis and Investigation of Chitosan Derivatives Formed by Reaction with Acyl Chlorides', Journal of Carbohydrate Chemistry, 24: 1, 41 — 54
To link to this Article: DOI: 10.1081/CAR-200049412
URL: http://dx.doi.org/10.1081/CAR-200049412

Journal of Carbohydrate Chemistry, 24:41–54, 2005

ISSN: 0732-8303 print
DOI: 10.1081/CAR-200049412

Synthesis and Investigation of Chitosan Derivatives Formed by Reaction with Acyl Chlorides

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Chitosan is a linear polysaccharide obtained from chitin deacetylation. Several applications of chitosan have been proposed in specialized literature: water treatment, cosmetic, food additives, development of biomaterials, and mainly drug delivery. In the present paper chitosan was hydrophobized by esterification reaction with acyl chlorides; its derivatives were swelling in water and characterized by ^{13}C- and ^{1}H-NMR and infrared spectroscopy. The main scope of this result was to generate hydrophobic derivatives through reactions on hydroxyl and amine groups of polysaccharides by incorporation of long alkyl chains. In accordance with the results, the exponential growing of degree of substitution (DS) with TEA concentration shows the necessity of higher concentrations to obtain good results. In the presence of acyl chloride the DS achieved higher values for lower concentrations of this, due to the presence of a favorable amount of TEA.

Keywords Chitosan, Esterification, Drug delivery systems, NMR, Infrared spectroscopy

INTRODUCTION

A considerable number of drug delivery systems have been investigated, for example, liposomes,[1] nanoparticles,[2] and microspheres.[3] Implants made from biodegradable polymers[4] and hydrogels[5] are two other important applications. In particular, drug delivery systems from biodegradable polymers have been extensively studied owing to two important biologic properties: biocompatibility and high capacity of degradation, in a specific context.[6,7]

Received November 5, 2004; accepted November 29, 2004.
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Chitin[8] is a polysaccharide usually found in crustacean shells of crabs, and shrimp or in the walls' fungi cells, but its insolubility in many solvents results in a small number of applications. The main derivative of chitin is chitosan, obtained by a process of deacetylation, which is soluble in aqueous acidic medium.

Chitosan (Fig. 1) is a linear polysaccharide of $\beta(1 \rightarrow 4)$-linked 2-amino-2-deoxy-D-glucopyranose that, recently, has received attention in many biologic procedures as a functional polymer because of several useful properties. These properties include nontoxicity, biocompatibility, biodegradability, antimicrobial activity, and chemical reactivity.[9] In a different view, many industries such as food processing, cosmetics, waste management, water clarification, wound healing, tissue repair, drugs, and gene delivery use this material as a basis of their products.[10]

Different chitosan derivatives have been developed, based on various reaction ways. In literature, chitosan-thioethylamidine derivative was prepared by imidoester reaction with isopropyl-S-acetylthioacetimidate.[11] Chitosan attached to hydrophobic alkyl groups and hydrophilic lactose has been gotten and studied for swelling measurements.[12] Either with carboxylic acid anhydrides or with acid chlorides, acylation has been used to proceed at the free amino and hydroxyl groups.[13] In this way, water-soluble derivatives have been performed with acetic acid and dicyclohexylcarbodiimide;[14] acetyl and nonanoyl derivatives has been gotten in pyridine with acyl chorides;[13] acylated or carbamoylated derivatives were prepared with DMAc containing LiCl;[15] acylated derivatives were performed in pyridine/chloroform with carboxylic acid chlorides;[16] and acylchitosan has been prepared by chemical N-acylation of the chitosan with the corresponding fatty acid anhydride.[17,18] In a general way, acylation reactions are lead frequently in aqueous acetic acid/methanol.[19] The free amino group of chitosan reacts with an aldehyde to give the correspondent Schiff base. Long alkylidene groups were introduced efficiently with aliphatic aldehydes.[20]

In a more general way, polysaccharides have been modified to obtain amphiphilic polymers capable of forming micellar structures and to dissolve

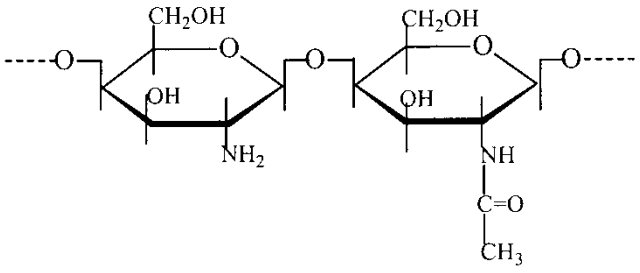


Figure 1: Chemical structure of chitosan.

organic molecules in the hydrophobic domain.[21,22] Recently, amphiphilic polysaccharides have been used to modify the surface of nanospheres and microparticules in drug delivery systems. In this context, the hydrophobic domains can be studied using properties of the photophysics probes.[4,23–25]

With the perspective of real applications, the present article investigates a method for hydrophobization of the chitosan by reaction with stearoyl and lauroyl chloride and the characterization of their products. This method has as a general idea to generate hydrophobic derivatives through reactions on hydroxyl and amine groups of polysaccharide by incorporation of long alkyl chains. The possibility of the use of these derivatives as hydrophobic models of drug delivery depends on the degree of substitution (DS) and solubility of them; therefore, these characteristics were studied. In accordance with the results, the exponential growing of the DS with the TEA concentration shows the necessity of higher concentrations to obtain good results. In the presence of acyl chloride, the DS achieved higher values for lower concentrations of this, due to the presence of a favorable amount of TEA.

EXPERIMENTAL

Chemicals

Chitosan with degree of deacetylation (DDA) ranging from 75–85% and average molecular weights of 50,000 were purchased from Aldrich Chemical. Stearoyl (99%) and lauroyl (98%) chlorides were purchased from Aldrich Chemical and Acros Organics, respectively. Triethylamine (TEA, 99%) and KBr (99 + % FTIR) were purchased from Acros Organics and Aldrich Chemical, respectively. Other chemicals were reagent grade and used without further purification.

Synthesis of Chitosan Derivatives

Chitosan 1% (w/v) was dissolved in aqueous solution of glacial acetic acid 1% (v/v) under stirring.[26] This solution was taking to the reaction vessel, which was placed in a constant temperature bath ($18 \pm 0.01^{\circ}C$) where it remained for 1 hr after addition of the TEA and the acyl chloride, always under continuous stirring. The polysaccharide solution was submitted to a double precipitation in 200 mL ethanol, filtered, and washed with ethanol and hexane. The derivative was transferred to a soxhlet system and extracted with hexane to ensure the elimination of nonbound acyl chloride. The DS was controlled by the acyl chloride or TEA amount. The ratios of chitosan (Q), stearoyl or lauroyl chloride (Ch), and TEA are shown in Table 1.

Table 1: Molar ratios for the derivatives of chitosan with chitosan (Q), acyl chloride (Ch), and TEA amounts.

Stearoyl derivatives	Molar ratio Q:Ch:TEA	Lauroyl derivatives	Molar ratio Q:Ch:TEA
TQ-S1	1.0:1.0:1.7	Q-L1	1.0:1.0:6.7
TQ-S2	1.0:1.0:4.2	Q-L2	1.0:0.70:6.7
TQ-S3	1.0:1.0:5.0	Q-L3	1.0:0.33:6.7
TQ-S4 = Q-S1	1.0:1.0:6.7	Q-L4	1.0:0.15:6.7
Q-S2	1.0:0.70:6.7	Q-L5	1.0:0.10:6.7
Q-S3	1.0:0.33:6.7		
Q-S4	1.0:0.15:6.7		
Q-S5	1.0:0.10:6.7		

Characterization of Chitosan Derivatives

NMR Spectroscopy

^{13}C- and ^{1}H-NMR measurements were performed on a DRX400 Bruker NMR spectrometer at 400 MHz and 80°C, using TMS (tetramethylsilane) as internal reference. For those measurements, 10 mg of the polysaccharide samples were dissolved in 1 mL of D_2O/HCl (100:1 v/v) after continuous stirring for 18 hr. ^{1}H-NMR measurements also were used to determine the DS.

FT-infrared (IR) Measurement

Infrared spectra samples were obtained by Fourier Transform Infrared Spectroscopy (FT-IR, Spectrum 2000 Perkin Elmer). The samples were prepared in thickness KBr pellets (5 mg in 200 mg of KBr) and stabilized under controlled relative humidity before acquiring the spectrum. For each sample 30 scans were recorded from 4000 to 500 cm^{-1} with a resolution of 4 cm^{-1}.

Swelling Test

The swelling index of chitosan derivatives was calculated by the following equation

$$Swelling\ index, \% = \frac{W_s - W_o}{W_o} \times 100 \quad (1)$$

where W_o is the weight of a dried chitosan derivative and W_s is the weight of a swollen derivative after the immersion in water at time t.

Solubility Test

Derivatives of chitosan (1 mg) were placed in a tube with each of solvent (1 mL). After mixing with an ultrasonicator, the mixture was stored at room temperature for 7 days and visually observed.

RESULTS AND DISCUSSION

Characterization of Chitosan Derivatives

FT-IR spectroscopy was used to confirm the chemical structure of chitosan derivatives. Figure 2 gives the spectra of chitosan and its lauroyl and stearoyl derivatives. In FT-IR, the spectrum of chitosan can be observed by the absorption bands at 2900 cm^{-1}, attributed to the C-H stretching, and at 3400 cm^{-1} due to the terminal hydroxyl groups. Some specific peaks of this polysaccharide are amide I ($\sim$1700 cm^{-1}), amide II ($\sim$1585 cm^{-1}), and amide III ($\sim$1320 cm^{-1}) in agreement with the literature.[27–29]

The lauroyl and stearoyl derivatives spectra, with different DS can be viewed in Figure 2 for Q-L1 and Q-S1. The region 2900–2800 cm^{-1} was modified, and this is a consequence of the input of several groups of -CH_3 in the structure of chitosan after the substitution reaction. The appearance of a carbonyl band at $\sim$1800 cm^{-1} corresponding to the ester group[27] formed in the esterification reaction, which was absent in the native chitosan, can be observed. The peak of amide I was intensified and dislocated of $\sim$40 cm^{-1} at

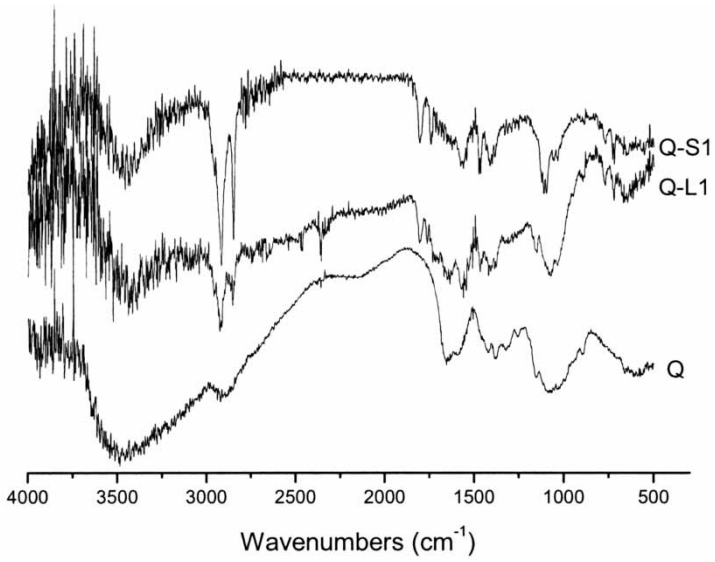


Figure 2: FT-IR spectra of chitosan (Q) and its derivatives, Q-L1 and Q-S1.

the same time that the peak of amide II was decreased, suggesting a higher pseudo degree of acetylation. These new characteristics are more intensified with the increase of acyl chloride (Table 1, Q-S5 to Q-S1) or TEA concentration (Table 1, TQ-S4 to TQ-S1).

The chemical shifts of the ^{13}C-NMR signals of chitosan are given in Figure 3 and show: δ(C-1) = 95.01, δ(C-2) = 53.60, δ(C-3) = 67.61, δ(C-4) = 74.78, δ(C-5) = 72.28, and δ(C-6) = 57.99 ppm in agreement with the literature.[30–32] Moreover, little signals at ~98.00 and ~20.0 ppm that correspond to the acetyl residue of chitosan can be observed. The ^{13}C-NMR of all derivatives confirms the chemical modification, as exemplified to Q-L1 and Q-S1 derivatives (Figure 3). This figure shows an increased signal at 174 ppm (carbonyl carbon)[31] when compared with native chitosan; signals at 8.00, 18.00, and 27.00 ppm attributed to the -CH3 group and the two -CH2- of substituent; and a signal at 45.00 ppm attributed to the -CH2- group near to carbonyl. Besides, some contribution of acetyl residue of chitosan at 23.00 ppm can be observed. The signals are in agreement with the literature[33] and with the relative shifts predicted to long alkyl chains (10–30 ppm, -CH_3 group and 15–55 ppm, -CH_2 group).[34] As well as in measurements of FT-IR, the new characteristics are influenced by the increase of acyl chloride and TEA concentrations.

It is well known that the modification of OH groups in polysaccharides entails both a downfield shift of the carbon bearing the OH group itself (α-effect) and an upfield shift of the adjacent carbon (β-effect) with respect to carbons with unsubstituted OH group.[34–36] In accordance with this, the downfield shift for all carbons in the spectrum, except C-4, as exemplified to stearoyl derivatives (Table 2) can be observed however, the α-effect only can

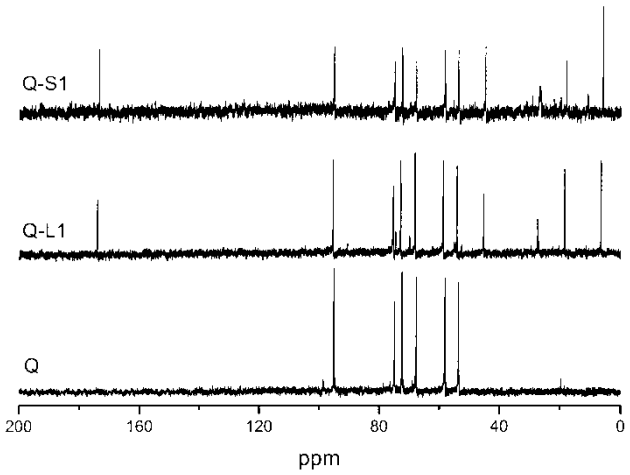


Figure 3: ^{13}C-NMR spectrum of chitosan (Q), lauroyl-derivatives (Q-L1), and stearoylderivative (Q-S1).

Table 2: ^{13}C-NMR assignment of the field shift for carbon of OH group substituted: chitosan ($\delta_{chitosan}$) and derivative Q-S1 ($\delta_{Q\text{-}S1}$).

	$\delta_{chitosan}$ (ppm)	$\delta_{Q\text{-}S1}$ (ppm)
C-1	95,01	95,47
C-2	53,60	54,09
C-3	67,61	68,06
C-4	74,78	73,31
C-5	72,38	72,45
C-6	57,99	58,70

be related with the substitution on the OH groups of C-3 and C-6. Besides, the presence of acetamido groups cause the downfield shift of the ^{13}C-NMR spectrum of chitin shifted with respect to chitosan, which seems to be related to the acetamido groups[31] and therefore is in accordance with FT-IR data that suggest an increase of the pseudo degree of acetylation.

The chemical shifts of the ^{1}H-NMR signals of chitosan are given in Figure 4a and show: $\delta = 5.48$ (H-1, deacetylated), $\delta = 5.19$ (H-1, acetylated), $\delta = 4.90-4.00$ (H-2, acetylated, H-3 to H-6), $\delta = 3.8$ (H-2, deacetylated), $\delta = 2.60$ ppm (-CH_3, acetamido group) ppm in agreement with the literature.[26,29,32,37]

The degree of acetylation (DA) of native chitosan was determined from the integral intensity of methyl protons from the acetamido group by relating them to the integral intensity of the bonded proton to the carbon 2 in the glycosidic

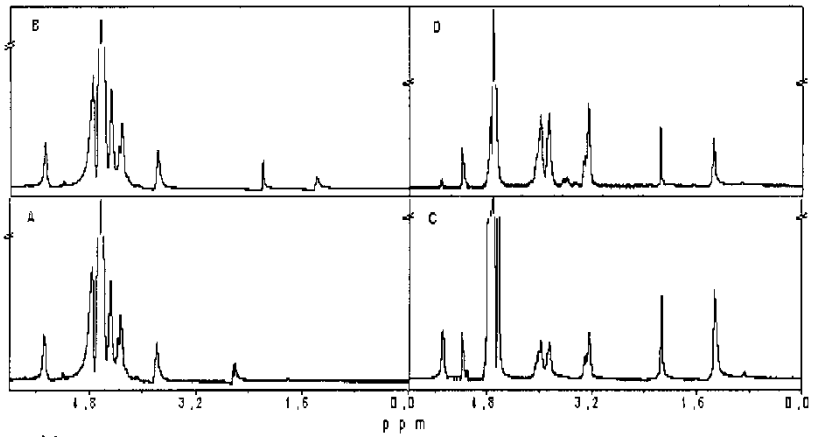


Figure 4: ^{1}H-NMR spectra of (A) chitosan and lauroyl-derivative: (B) Q-L5, (C) Q-L3, and (D) Q-L1.

ring. In this way, the degree of deacetylation (DDA) of 87.2% is in agreement when compared with commercial information.

The spectral analysis of the ^{1}H-NMR data confirms the substitution in the chitosan, as exemplified to the lauroyl derivatives. Figure 4b–d shows a comparative ^{1}H-NMR spectrum of derivatives (Q-L5, Q-L3, Q-L1) and of the nonmodified chitosan. In this figure new peaks and a modification in the spectrum as a consequence from a different electronic environment is shown.[38] Two peaks at 2.1 and 3.1 and the peak at 1.5 ppm are attributed to the -CH_2 and -CH_3 groups of substituent, respectively, which are the protons of the alkyl chain of the hydrophobic moiety.

Special attention must be given to the chemical shifts of H-1 (deacetylated) and H-1 (acetylated): The increase of substitution results in a relative decrease of the peak intensity in the first one and an increase in the second one, confirming the assumption of the reaction on the amine group of chitosan. In fact, the synthesis generated derivatives with a bigger pseudo degree of acetylation. This behavior follows the increase of acyl chlorides or TEA concentrations.

The DSs were estimated by ^{1}H-NMR measurements (Figures 5 and 6). The values were obtained from the integral intensities of methyl protons from the substituent by relating them to the integral intensities due to both H-1 protons. Figures 5 and 6 show the variation of the DSs with acyl chloride or TEA, plotted for stearoyl and lauroyl derivatives. In Figure 5 the logarithmic growing shows that for higher concentrations of acyl chloride the values of DS not changed significantly. The analysis of data show that the DS for the lauroyl derivatives in high concentrations are bigger when compared with the values of stearoyl derivatives. Due to the similarity of chemical between both chlorides and in agreement with the literature,[12]

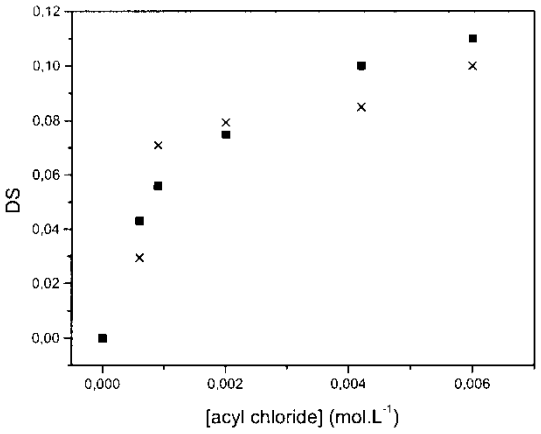


Figure 5: Variation of the degree of substitution (DS), measured by ^{1}H-NMR spectra for the stearoyl derivatives (×) and lauroyl derivatives (■) *versus* the acyl chloride concentration.

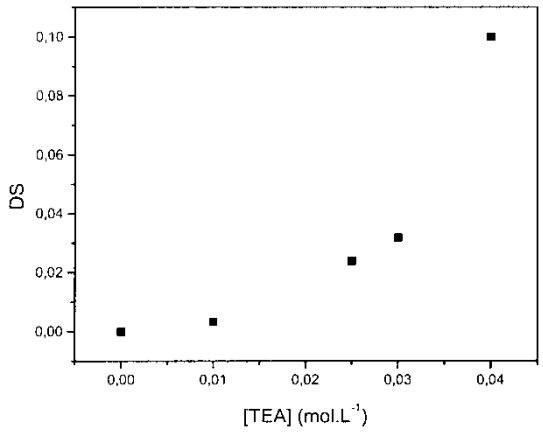


Figure 6: Variation of the degree of substitution (DS), measured by ^{1}H-NMR spectra for the stearoyl derivatives of chitosan *versus* the TEA concentration.

these results suggest a reaction more favorable when lower is the length of the substituent chain.

The amount of TEA present in the reaction environment seems to be a factor that controls the reaction kinetic. In Figure 6 the exponential growing shows the importance of higher concentrations of TEA to obtain higher values of DS. Similar results have been described for the esterification reaction of dextran with *p*-hexylbenzoyl chloride.[39,40] These results seem related with the availability of TEA to receive the proton of the esterification reaction.[39]

Swelling Test

The swell of polymers as well as their absorbing capacity and capillary lead to a considerably strong adhesion. These are important properties of drug delivery systems; therefore, a prolonged residence time of the system on mucosa leads to an extended period of absorption and consequently of improved bioavailability.[41] Slow swelling is required to avoid the formation of an overhydrated form that loses its mucoadhesive properties before reaching the target. Thus, the swelling behavior of chitosan derivatives having various DS was investigated. Figure 7 shows that the swelling index of chitosan derivatives decrease with the increase of the DS, in agreement with the literature that described that the swelling index is positively affected by the presence of hydrophilic groups.[42]

The biggest value of swelling index for less substituted chitosan when compared with the more substituted can be attributed to a tighter and more compact structure for limiting water uptake.

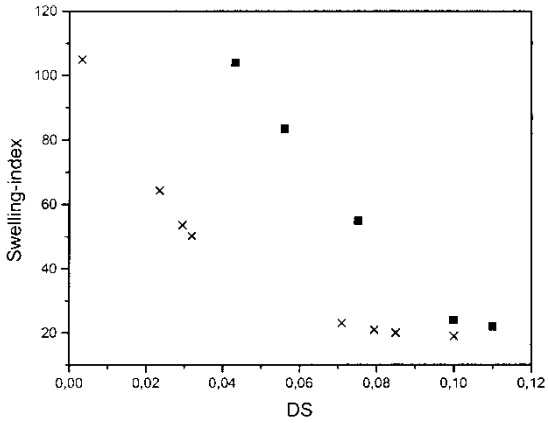


Figure 7: Swelling index *versus* degree of substitution (DS) of stearoyl (×) and lauroyl derivatives (■).

Solubility

Chitosan is insoluble in both water and organic solvents. It is soluble in aqueous diluted acids, dissolving in hydrochloride acid and aqueous organic acids such as formic and acetic. Chitin shows limited affinities for solvents and is soluble in special solvents such hexafluoro-acetone.[9] Because alkyl groups reduce the solubility of chitosan, derivatives were insoluble in water. The derivatives soluble in 2% aqueous acetic acid were in the DS ranges of <0.071 for both alkyl chains. The derivatives soluble in hexafluoroacetone were in the DS ranges of <0.079 for both alkyl chains, showing solubility diminished with relation to chitin (Table 3).

In the aqueous solution, the hydrophobic groups are present in the inert part of chain clusters, and the hydrophilic amino groups are present in the other part; the proportion of the hydrophobic and hydrophilic groups controls their solubility. These results might suggest that the hydrophilic-hydrophobic balance of the entire molecule was essential for their water insolubility.

Reaction Mechanism

The expectation in this work was to promote the esterification by the nucleophilic reaction of both amine and hydroxyl groups of polysaccharide on the carbonyl group of the acyl chloride. In this spirit, the objective was to generate a range of possibility for the introduction of the hydrophobic groups.

In Scheme 1, the arrow 1 indicates a step of synthesis relative to the nucleophilic reaction of the hydroxyl groups on the carbonyl group of acyl chloride. The TEA forbids an acid reaction environment, which will cause the carbonyl group to be protonated and more reactive, and therefore cause an uncontrollable

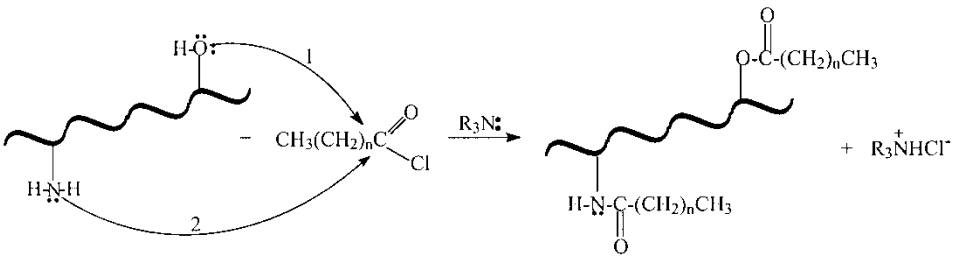
Table 3: Solubility for the chitosan derivatives, ambient temperature, where I (insoluble) and S (soluble).

	Solubility		
Solvent	**2% Acetic acid**	**Hexafluoro-acetone**	**Water**
Stearoyl derivatives			
TQ-S1	S	S	I
TQ-S2	S	S	I
TQ-S3	S	S	I
Q-S1 (TQ-S4)	I	I	I
Q-S2	I	I	I
Q-S3	I	S	I
Q-S4	S	S	I
Q-S5	S	S	I
Lauroyl derivatives			
Q-L1	I	I	I
Q-L2	I	I	I
Q-L3	I	S	I
Q-L4	S	S	I
Q-L5	S	S	I

reaction. Besides, the amine (TEA) must have a structure unfavorable sterically to not react with the carbonyl group.

The ^{13}C-NMR measurements suggest that reactions on hydroxyl groups of C-3 and C-6 carbons can be occurring. The $-CH_2-OH$ group is an alcohol primary, most sterically accessible and reactive. However, the $-OH$ group on C-3 seems also to participate to the reaction, probably in lower percentage.

The arrow 2 indicates a step of synthesis relative to the nucleophilic reaction of the amine groups on the carbonyl group of acyl chloride. The nucleophiles have a couple of free electrons; therefore, its reactivity is related with intensity of force as the Lewis basis.[43]



Scheme 1: Reaction route for the synthesis of modified chitosan, where R_3N is the TEA, and n has the 10 or 16 values for the lauroyl or stearoyl chlorides, respectively.

From the ^{13}C-and ^{1}H-NMR and infrared spectroscopy measurements was possible to visualize the input of functional groups in the chitosan molecule and the success of the synthesis purpose.

CONCLUSION

A method for hydrophobized chitosan by reaction with acyl chlorides was shown. From ^{13}C- and ^{1}H-NMR and infrared spectroscopy was possible to confirm its success.

This method is suitable for the transformation of chitosan into hydrophobic derivatives, which can be achieved through the control of DS.

Good drug delivery systems were able to swell rapidly and retain large volumes of water in their swollen structure without dissolution. For this reason, both low values of DS and little solubility of drug delivery systems are required to take the drug to the specific place. In this way, the solubility and swelling values show that the derivatives are expected to be good models of drug delivery systems.

ACKNOWLEDGMENT

The author thanks FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for financial support.

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