Synthesis and Characterization of Chitosan Alkyl Carbamates

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ABSTRACT: The synthesis of chitosan methylcarbamate (ChMC) and ethylcarbamate (ChEC) is described by using a new methodology. Polymers with substitution degrees up to 63% for ChEC and 68.5% for ChMC were obtained. Derivatives with lower substitutions were acid soluble but those with higher ones were completely insoluble. This could be due to the loss in hydrophilic sites when the substitution degree increases. The reaction conditions and degree of substitution obtained for both derivatives were also de-

scribed. A complete chemical characterization was carried out by spectroscopic techniques. The thermal degradation of chitosan and derivatives were studied in the range 25–500°C and both derivatives were shown to be thermally less stable than chitosan. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 86: 2742–2747, 2002

Key words: chitosan; chitosan derivative; thermogravimetric analysis; NMR; biopolymer

INTRODUCTION

Chitin is a natural polymer that can be obtained from crustacean shells where it is associated with inorganic salts, proteins, lipids, and pigments.[1] Chitosan, a poly(β-(1–4)-D-glucosamine), can be obtained by deacetylation of chitin with concentrated alkali or enzymes.[2]

Several N-derivatives of chitosan were synthesized by taking advantage of their biocompatibility[3] and biodegradability.[4] From a chemical point of view, these modifications were done to make them soluble in water or organic solvents[5] and to improve their natural metal chelating properties.[6] Regarding their biological activity, the main objectives were to improve their fungicidal[7] and bactericidal[8] properties.

Carbamates are widely used in agriculture because of their known insecticide, fungicide, and herbicide activity. The main chemical characteristic of these compounds is the presence of the —NH—CO—O—[9] group in their structure.

Aiba, in 1993,[10] synthesized chitosan methyl and ethylcarbamate by using an anhydride as a carbamilic reagent. He studied the relation between the substitution degree with the initial reagent employed and proposed a method to find the substitution degree by using FTIR spectroscopy. However, further spectroscopic studies or thermal degradation of the polymers were not carried out.

The purpose of this work was to synthesize and characterize, by using spectroscopic and thermal techniques, chitosan methyl and ethylcarbamate obtained by new methodology. If we consider that most of the biocides used in the crop damage control and management have produced some environmental problems, these new chitosan carbamates could be a good alternative to be used as biocides, because they have a biodegradable matrix and could possess fungicidal and/or insecticidal properties.

EXPERIMENTAL

The ethyl and methyl chloroformate are of reagent grade and were purchased from Aldrich, Milwaukee, WI.

The chitosan samples were obtained from chitin isolated from the red lobster (*Pleuroncodes monodon*) caught off the Chilean coast. The molecular weight of chitosan was determined in an Ostwald capillary vis-

TABLE I
Reaction Conditions and Degree of Substitution for Chitosan Ethyl and Methylcarbamate

Sample	Ratio (reagent/chito)	Reaction time (h)	Degree of substitution (DS %)[a]	Degree of acetylation (DA %)[a]
ChEC	1 : 1	12	23.1	16
	2 : 1	12	31.3	16
	5 : 1	12	52.4	16
	10 : 1	12	63	16
ChMC	1 : 1	12	26.8	16
	5 : 1	12	55.1	16
	10 : 1	12	68.5	16

ChEC, chitosan ethylcarbamate; chito, chitosan; ChMC, chitosan methylcarbamate.
[a] Obtained by ^{1}H-NMR or elemental analysis.
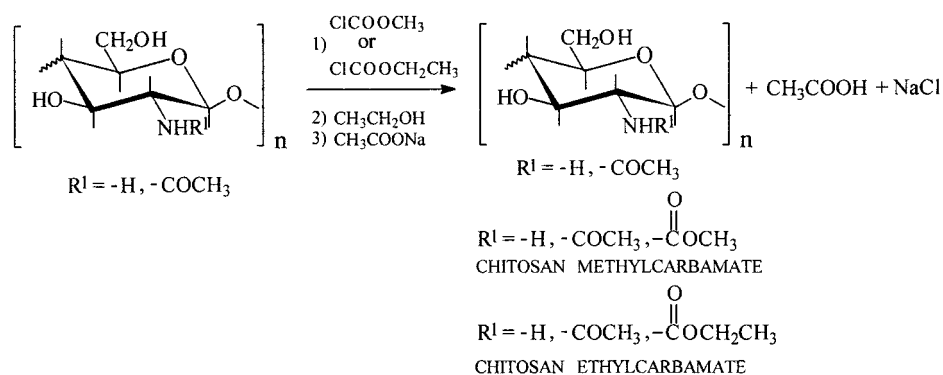

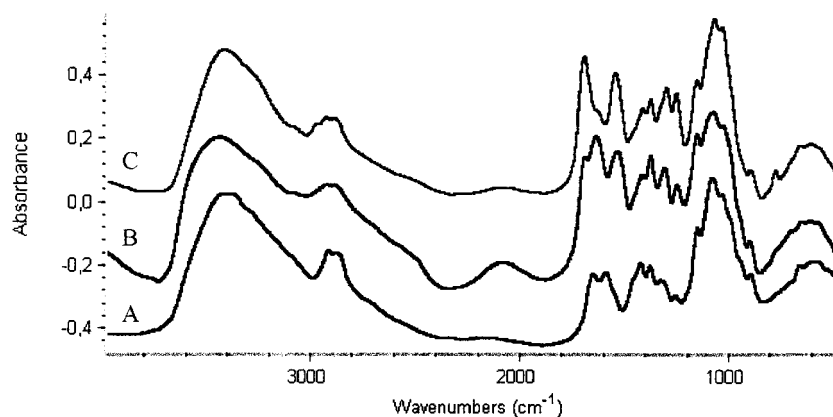**Figure 1** Reaction scheme to obtain carbamate derivatives of chitosan.

Figure 2 FTIR spectra of chitosan (A), ChEC DS 31.2% (B), and DS 52% (C).

cometer by using the solvent system CH_3COOH $0.3M$/CH_3COONa $0.2M$ and the acetylation degree (DA) by ^{1}H-NMR.[2] The sample employed in this work has a DA of 16% and $M_v = 81,250$ g/mol.

Chitosan methyl (ChMC) and ethylcarbamate (ChEC) synthesis

The chitosan (1 g) was placed in a round-bottom flask and different concentrations of methyl or ethylchloroformate (see Table I) in ethanol were added under stirring. After 30 min, a corresponding volume of so-dium acetate $0.05M$ was added and the pH adjusted to 3. The mixture was stirred for 12 h at room temperature and then the solution was neutralized. The product was separated by centrifugation, washed three times with acetone, and dried in a vacuum oven at 40°C for 4 h. When the product was soluble in water, it was dissolved and dialyzed for 3 days and then lyophilized.

The ^{1}H- and ^{13}C-NMR spectra were recorded on an AC 300 Bruker spectrometer equipped with a process controller, an ASPECT 3000 computer, and a variable temperature system. The temperature was 335 K. For
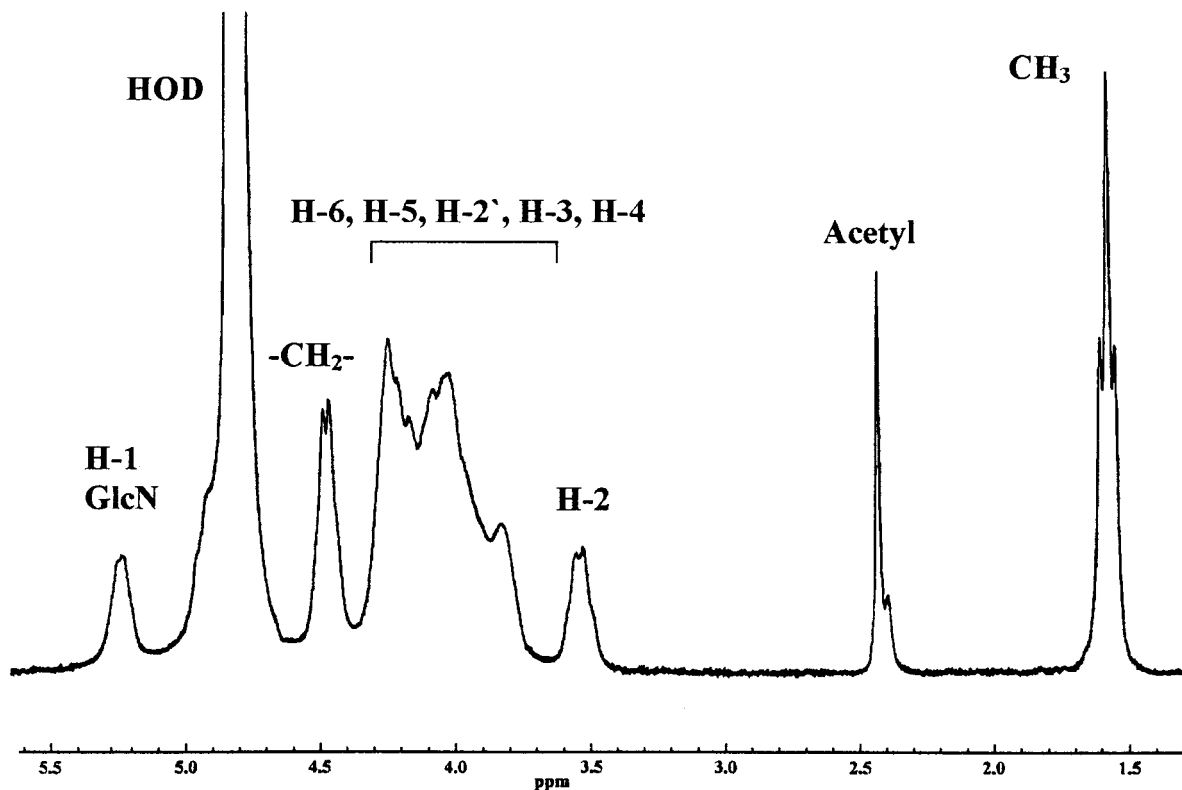
Figure 3 ^{1}H-NMR spectra of chitosan ethylcarbamate.

NMR measurements, the solutions were dissolved in D_2O, freeze dried to displace adsorbed moisture, and then dissolved in the same solvent. The sample concentration was 10 g/L in D_2O (99.9%) for ^{1}H-NMR and 100 g/L for ^{13}C-NMR experiments. 2,2-dimethyl-2-silepentene-S-sulfonate (DSS) was used as a reference for ^{1}H-NMR experiments and tetramethylsilene (TMS) for ^{13}C-NMR experiments.

Elemental analysis: C, H was carried out with Heraeus equipment; N was determined by Kjeldahl method.

Infrared spectra were measured by using a FTIR Nicolet Magna 5PC spectrophotometer coupled to a PC with OMNIC software for data analysis. The KBr disks were prepared by blending KBr well with dried polymer at 2% concentration. Spectra were recorded at a resolution of 4 cm^{-1} and 64 scans were accumulated.

A Perkin–Elmer Model TGA-7 thermogravimetric system, with a microprocessor-driven temperature control unit and TA data station, was used. The sample weight was recorded and generally ranged between 3 and 5 mg. The sample was placed in the

balance system and the temperature was raised from 25 to 550°C under nitrogen atmosphere at a heating rate of 10°C/min. The sample weight was continuously recorded as a function of temperature.

RESULTS AND DISCUSSION

The reaction conditions employed in this work and the products obtained are presented in Figure 1.

Chitosan in a solid form was reacted with methylchloroformate to obtain ChMC and with ethylchloroformate to synthesize ChEC. When sodium acetate was added to the reaction system, it was convenient to add some drops of NaOH 1M to raise the pH value up to 3. This allowed us to partially neutralize the acetic or hydrochloric acid formed during the reaction, thus regenerating the acetate media. Such acids are undesirable because they could protonate chitosan, thus destroying the possibility of substitution by the nitrogen atom. The conditions and degree of substitution (DS) and DA obtained for each derivative are shown in Table I.

TABLE II
^{1}H-NMR Chemical Shifts (ppm) for Chitosan Ethylcarbamate

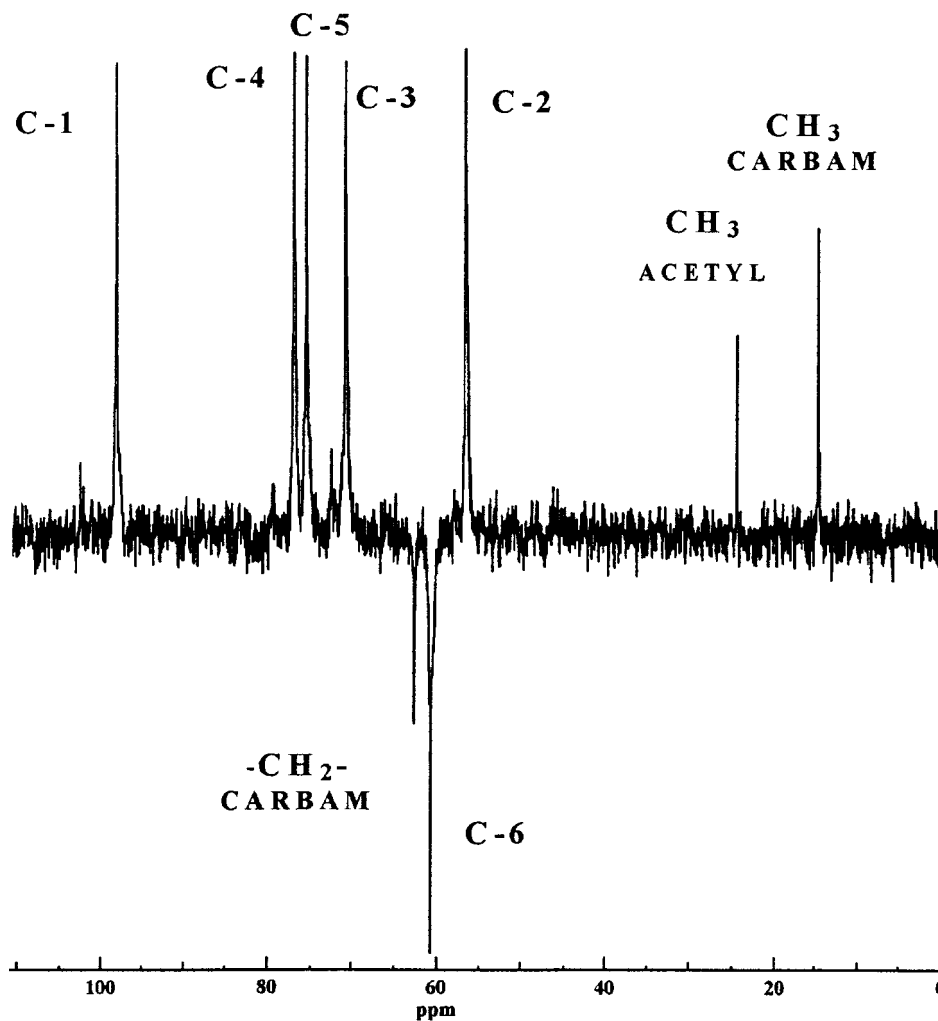| [1]H	CH_3 carbam	CH_3 acetyl	H-2	O-CH_2 carbam	H-6, H-5, H-4, H-3, H-2 acetylated units	H-1 acetylated units
ChEC	1.57	2.42	3.52	4.46	3.7–4.4	5.23

Figure 4 The ^{13}C-DEPT 135 NMR spectra of chitosan ethylcarbamate.

It was observed that no deacetylation occurred during this process and the maximum degree of substitution obtained is 68.5% for ChMC and 63% for ChEC. It is worth noting that, for the same reagent/chitosan concentration ratio, a slightly higher substitution degree was obtained for ChMC, agreeing with the higher reactivity of methylchloroformate. Both derivatives at lower substitution degrees were soluble in acidic media but the amount of insoluble products increased along with the substitution degree, probably because of the decrease of the hydrophilic sites in chitosan. In fact, ChMC possessed higher insolubility than the ethyl derivative at lower substitution. This could be due to the chitinlike structure it possesses (see Fig. 1), where intra- and inter-rhydrogen bonding rearrangement in solid state may occur preferentially, rather than in the ethylcarbamate because of the presence of a smaller alkyl group.

FTIR spectroscopy

The products were characterized by FTIR. The chitosan and chitosan ethylcarbamate spectra at different substitution degrees are shown in Figure 2.

TABLE III
^{13}C-NMR Chemical Shifts (ppm) for Chitosan Methyl and Ethylcarbamate

^{13}C	CH$_3$ carbam	CH$_3$ acetyl	C–2	O–CH$_2$	C–6	C–5	C–4	C–3	C–1	C=O
ChEC	17.3	25	58.3	63.4	61.7	75.5	77.9	71.8	101.2	180.3
ChMC	56	25.2	58.5	—	63.1	77.4	79.3	73.5	101.6	180.8

New absorption peaks are observed at 1700, 1256, and 778 cm^{-1}, which is in agreement with a previous report.[10] It could be observed that, when the degree of substitution increases, the Amide I band, corresponding to the stretching $\nu_{NH-CO-R}$ (1700 cm^{-1}), increases. At a lower substitution degree, it appears as a shoulder, and at a higher degree, it is a well-defined band which overlaps the Amide I band corresponding to $\nu_{C=O}$ vibration (1650 cm^{-1}) of acetyl groups in chitosan. The band Amide III at 1302 cm^{-1}, due to combination of NH deformation and ν_{CN} stretching vibration, and the peak at 778 cm^{-1} (Amide IV band), increase along with the substitution degree. The peak at 1256 cm^{-1} is due to the ν_{CO} stretching vibration of the carbamate bond. No evidence was obtained, indicating ester formation, and only carbamates were formed, indicating that the reaction was placed selectively by the N-position.

NMR spectroscopy

To better characterize the derivatives, a soluble sample of every polymer was dissolved in D$_2$O to register the proton and carbon spectra. Figure 3 shows the ^{1}H-NMR spectrum of ChEC, which is very similar to ChMC, except in the former the signal of O—CH$_2$ does not appear. All signal assignments were done by using chitosan spectra published by other authors as a reference.[2] The methyl signal belonging to methylcarbamate appears as a singlet at 2.35 ppm and the same signal appears as a triplet centered at 1.57 ppm for the ethyl derivative, corroborating that it is linked to a methylene group. On the other hand, the signal at 4.46 ppm in Figure 3 possesses a similar intensity ratio to a methyl group at 1.57 ppm, and for this it was assigned to a O—CH$_2$ carbamate bond.

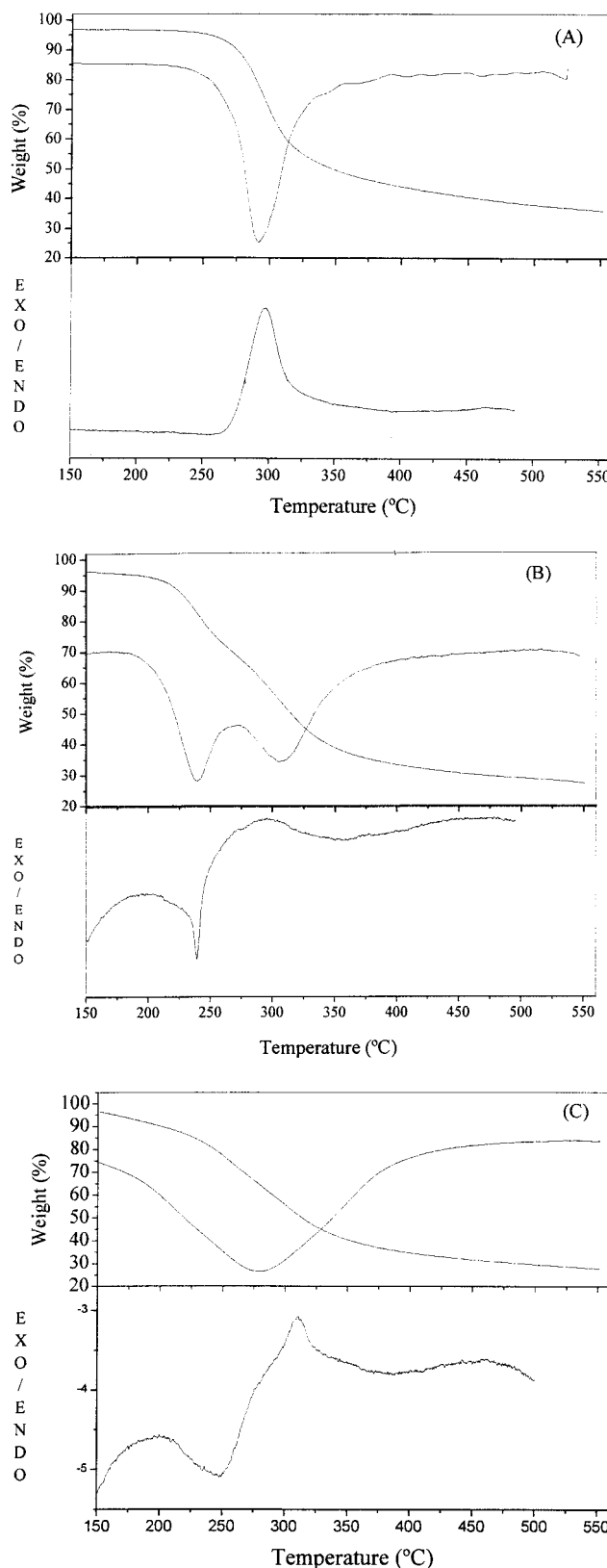The complete ^{1}H-NMR spectra assignment of chitosan ethylcarbamate is shown in Table II.

The ^{13}C-NMR spectra were also similar for both chitosan derivatives except the signal of O—CH$_2$ ethylcarbamate group, which does not appear for the methyl derivative, and the O—CH$_3$ signal of the last one that appears at 56 ppm. The ^{13}C-NMR spectrum of ChEC is shown in Figure 4.

Table III summarizes the chemical shift and the corresponding assignments for the ^{13}C-NMR of the ChEC derivative.

Thermal studies

In Figure 5(A–C), the thermogravimetry (TG), differential thermogravimetry (DTG), and differential scanning calorimetry (DSC) curves of chitosan and their derivatives, in nitrogen atmosphere, are presented. In all cases, the first part of the thermogram is not shown for temperatures lower than 150°C, in which the

Figure 5 The TG, DTG, and DSC curves of chitosan (A), chitosan ethylcarbamate (B), and chitosan methylcarbamate (C).

weight loss is due to the presence of water in the macromolecule.[11] Table IV summarizes the results of peak temperatures for different thermal effects and the associated enthalpy changes.

The chitosan DSC curve [Fig. 5(A)] first shows an exothermic effect in the range 220–420°C, with a maximum degradation rate at 295°C associated with a weight loss of 53.6%. This effect was attributed to a complex process corresponding to the first pyrolysis stage. During this process, simultaneous ring dehydration, depolymerization, and decomposition of acetylated and deacetylated units of the polysaccharide occur.[12] It was demonstrated for chitosan that the energy associated with this process is dependent on the acetylation degree of the polymer. The amount of energy evolved in this process increases when the acetylation degree of chitosan decreases.[13,14]

In general, the thermograms of both derivatives show less thermal stability than chitosan [Fig. 5 (B, C)]. In DSC curves, a first exothermic effect appears in the temperature range 190–273°C, peaking at 240°C for ChEC (ΔH 40.6 Jg^{-1}) and 248°C for ChMC (ΔH 65.4 Jg^{-1}). Such an effect is well defined for the ethyl derivative in TG-DTG curves, but to the contrary, for the ChMC polymer, it was not so clear. Because of the nature of this process and the temperature at which it occurs, it could be assigned to the decomposition of carbamate substituted units. The weight loss associated with ChEC (26.8%) was similar to ChMC (23.1%); however, the energy evolved in this process is 15.4 Jg^{-1} higher in ChMC. This difference could be account for by stronger bonds, due to higher internal rearrangement of the polymer chains, and explains the solubility behavior previously observed.

The second process for both derivatives is exothermic as in chitosan, ChEC ΔH -29.9 Jg^{-1} and ChMC ΔH -49.9 Jg^{-1}, but the released energy is somewhat lower than the former. This must be related to the decomposition of acetylated and deacetylated units that remain in the polymer. For ChEC, the peak appears at the same temperature as that of chitosan but the ChMC peak occurs at a higher temperature (16°C).

CONCLUSION

The synthesis of chitosan methyl and ethylcarbamate was possible by use of a new methodology. Both derivatives were characterized by combining solid and liquid state techniques. It was found that the

TABLE IV
Peak Temperatures in DSC Thermograms and Enthalpy Changes Associated with Chitosan and Derivatives

Samples	Temperature (°C)			ΔH (Jg^{-1})	Weight loss (%)
	Initial	Peak	Final		
Chitosan (A)	220	295	420	-300.1	53.6
ChEC (B)	190	240	273	40.6	26.8
	273	297	400	-29.9	34.7
ChMC (C)	200	248	273	65.4	23.1
	273	306	400	-49.9	32.4

solubility of the derivatives was strongly related to their substitution degree. The thermal analysis of the methylcarbamate shows stronger bonds than for the ethyl, which also explains their insolubility. Both derivatives are less thermally stable than chitosan. (Preliminary results have shown insecticide and fungicide properties for these derivatives, which are very important to prepare a slow release biocide.)

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