Synthesis and Characterization of New Arylamine Chitosan Derivatives

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ABSTRACT: The synthesis of new chitosan derivatives and their complete characterization by elemental analysis, Fourier transform infrared, thermogravimetric analysis, and cross-polarity/magic-angle-spinning ^{13}C NMR is described. A chitosan that was 96.5% deacetylated and had a viscosimetric molecular weight of 131,000 g/mol was prepared. *N*-(3,5-Diethylaminobenzoyl) chitosan with a degree of substitution of 29% and *N*-(4-ethylaminobenzoyl) chitosan with a degree of substitution of 60% were obtained. Such derivatives could be used as metal-chelating polymers, as flocculants, and in biomedical applications because of the aryl amine moieties in their structure. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 91: 807–812, 2004

Key words: thermogravimetric analysis (TGA); NMR; biopolymers

INTRODUCTION

Chitosan is chemically made up of 2-amine-2-deoxy-D-glucopyranose units β-(1,4)-linked. This biopolymer can be partially acetylated, but the extent of the acetylation is usually lower than 50% of its amine groups. For industrial applications, chitosan may be obtained by the chemical deacetylation of chitin isolated from crustacean shells.

In recent years, much attention has been paid to this polymer with respect to its biodegradability and biocompatibility. Furthermore, chitosan possesses reactive —OH and —NH_2, which can be derivatized.

Chitosan derivatization can serve various purposes, such as increasing the solubility of chitosan in water[1] or making it soluble in common organic solvents.[2] Different methodologies and synthetic strategies have been proposed in the literature. Among them, the synthesis of *O*-alkyl, *O*-acyl, *N*-acyl, *N*-arylidine, and *N*-alkylidene chitosan derivatives was reported by Hirano;[2,3] selective substitution by —OH or —NH_2 groups through the adjustment of reaction conditions, such as the pH, solvents, and temperature,[4,5] and the preparation of different compounds with alkyl and acyl halides[6,11] have also been mentioned.

Chitosan derivatives bearing groups such as amines,[12] phosphates,[13] acids,[14] thiourea,[15] sulfur,[16] amino acids,[17] and graft copolymers[18,19] have also been synthesized. All of them have been obtained to improve the metal retention capacity of chitosan.

In this sense, we reported[20] the synthesis of *N*-(2-hydroxy-3-methyl aminopropyl) chitosan, which increased the number of amino groups, and *N*-(2-hydroxy-3-mercaptopropyl) chitosan and 6-*O*-(mercaptoacetate)-*N*-(2-mercaptoacetyl) chitosan, which incorporated the mercapto groups.

The objective of this study was the synthesis and characterization of two new chitosan derivatives bearing arylamine moieties in their structure. These new derivatives were *N*-(3,5-diethylaminobenzoyl) chitosan (QDAB) and *N*-(4-ethylaminobenzoyl) chitosan (QAB). The treatment of water contaminated with metal ions or suspended materials, such as pigments, lipids, or proteins, with these derivatives can be achieved.

EXPERIMENTAL

Preparation of chitosan

Red lobster (*Pleuroncodes monodon*) shells were used as the raw material. The chitin was obtained by the treatment of the shells with 2*M* HCl at room temperature for 1 h followed by 3.5% NaOH at 70°C for 1 h.

The chitin was added to a 50% solution of NaOH placed in a 10-L glass reactor with mechanical stirring. The solid/liquid ratio was 1:15. The mixture was stirred at 500 rpm at 100°C for 90 min, and $NaBH_4$

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TABLE I
Assignment of Proton Signals in NMR in Solution

Proton	δ ppm (TMS)[a]	Intensity
H-1 of GlcN	5.32	0.700
H′-1 of GlcNAc	—	—
H-2 of GlcN	3.65	0.855
H′-2 of GlcNAc, H-3, H4, H5, and H-6	4.19–4.34	2.181
–CO–CH_3	2.5	0.048

[a] TMS = tetramethylsilane.

(10% w/v) was added to prevent oxidation and depolymerization. The final product was washed with hot water to eliminate NaOH and then was dried at room temperature.

Molecular weight of chitosan

The viscosimetric molecular weight was determined with the constants reported by Brugnerotto et al.[21] for the solvent system 0.3*M* CH_3COOH/0.2*M* CH_3COONa and with a degree of acetylation (DA) of 3.5%. The constants were $K = 0.076$ and $a = 0.76$, and a viscosimetric molecular weight of 131,000 g/mol was obtained. The degree of deacetylation (DD) was determined by ^{1}H-NMR to be 96.5%.

Synthesis of QDAB

To obtain this derivative, we reacted the amino groups of 3,5-diethylaminobenzoic acid with acetaldehyde to prepare the Schiff base. This intermediate product was dissolved in dimethyl sulfoxide (DMSO) and then reacted with chitosan at 100°C for 24 h. An excess of 2 mol of the Schiff base per mole of chitosan was used. The product was filtered, and the solid was washed successively with DMSO, acetone, ethanol, and water. Then, it was reduced with $NaBH_4$ in alkaline methanol at 50°C for 12 h to yield a secondary amine. The final product was washed with methanol and water and dialyzed with distilled water. Finally, the solid was filtered and dried in a vacuum oven at 60°C.

Synthesis of QAB

The procedure was the same as for QDAB, except that the starting reagent was 4-ethylaminobenzoic acid.

Infrared spectroscopy

Infrared spectra were measured on a Nicolet Magna 5PC Fourier transform infrared (FTIR) spectrophotometer (USA) coupled to a personal computer with OMNIC analysis software. Pellets were prepared by the blending of the polymer with KBr at a 2% concentration. Spectra were recorded at a resolution of 4 cm^{-1}, and 64 scans were accumulated.

Thermogravimetric analysis (TGA)

A PerkinElmer TGA-7 thermogravimetric system, with a microprocessor-driven temperature control unit and a TA data station (USA), was used. The masses of the samples were generally 2–3 mg. The sample pan was placed in the balance system equipment, and the temperature was raised from 25 to 550°C in a nitrogen atmosphere at a heating rate of 10°C/min. The mass of the sample pan was continuously recorded as a function of the temperature.

Elemental analysis

Carbon, hydrogen, and nitrogen microanalyses were performed with a PerkinElmer 2100 automatic analyzer.

^{1}H-NMR spectroscopy

The spectra were recorded on an Bruker AC 300 spectrometer equipped with a process controller (Germany), an ASPECT 3000 computer, and a variable-temperature system. The temperature was 335 K. For NMR measurements, the sample was dissolved in D_2O acidified with HCl, 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%), and 2,2-dimethyl-2-silapentene-*S*-sulfonate (DSS) was used as a reference for the ^{1}H-NMR experiments.

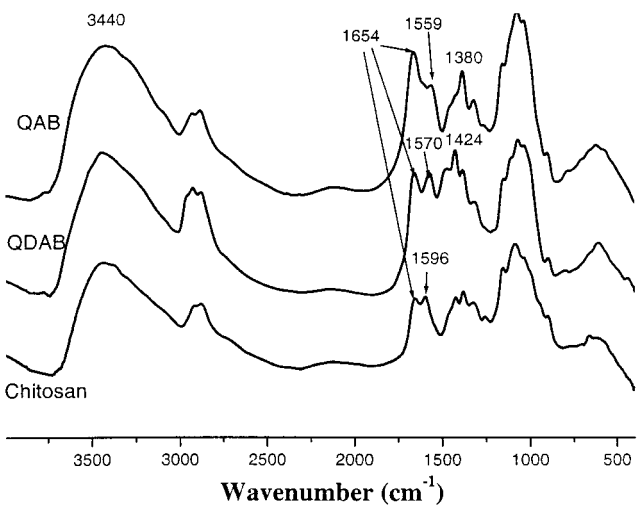


Figure 1 FTIR spectra of chitosan, QDAB, and QAB.

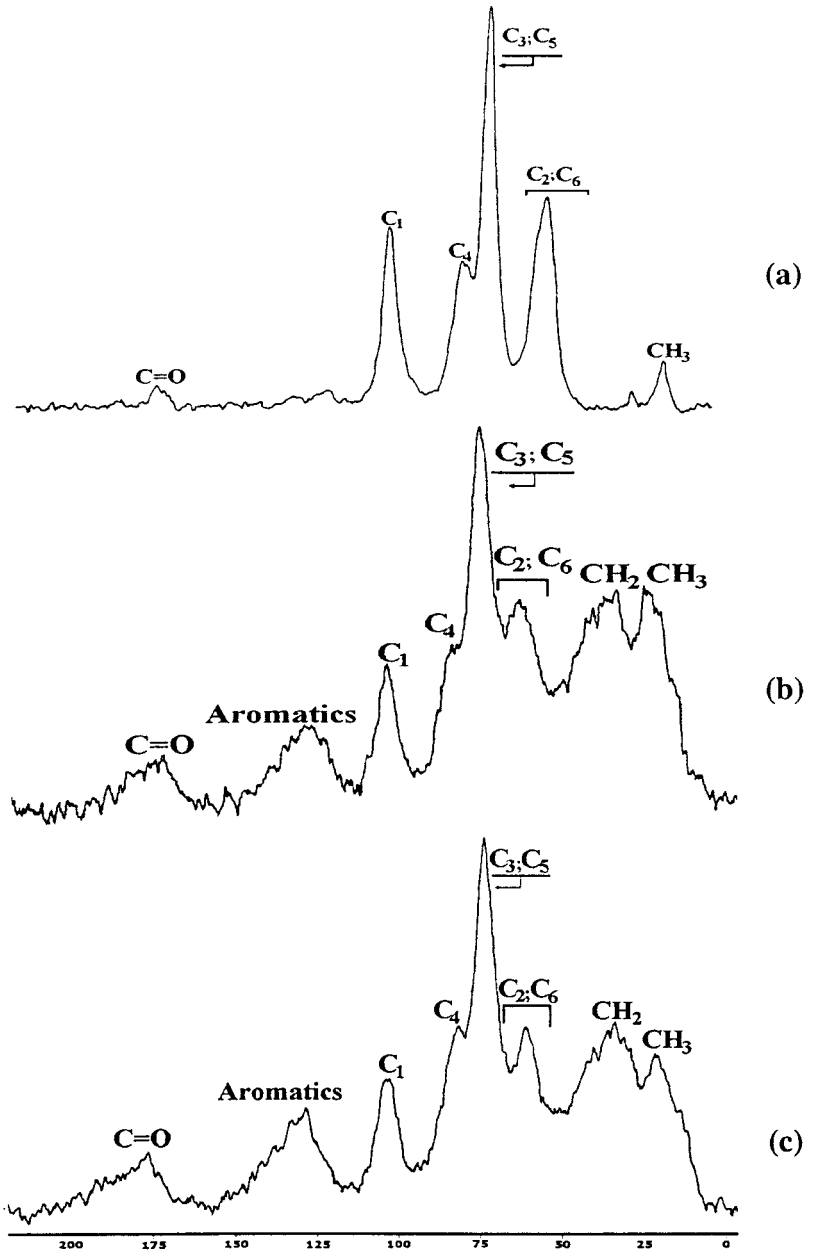


Figure 2 Solid ^{13}C-NMR spectra of (a) chitosan, (b) QDAB, and (c) QAB.

Solid-state ^{13}C-NMR

The cross-polarity/magic-angle-spinning ^{13}C-NMR spectra were recorded on a Bruker AMX300. In all cases, 3072 scans were accumulated. The contact time was 1 ms, and the repetition time was 5–50 ms. DSS was used as an external reference. The rotation speed varied from 3601 to 3639 Hz, depending on the sample.

RESULTS AND DISCUSSION

DA of chitosan

DA is one of the more important properties of chitosan because of its influence on the physicochemical properties of this polymer and its applications. The use of chitosan in metal retention is also influenced by this variable because the metal-ion retention takes place among free amino groups.

Different techniques have been suggested for evaluating DA of chitosan, among which FTIR,[22,23] ultraviolet spectrometry,[22,24] cross-polarity/magic-angle-spinning ^{13}C NMR,[21] elemental analysis,[25] circular dichroism,[26] and ^{1}H-NMR in the liquid state[21] may be mentioned.

In this work, ^{1}H-NMR was used for the DA determination because we considered it to be the most sensitive technique. The chitosan sample was prepared as mentioned before. The ^{1}H-NMR spectrum was measured, and the chemical shifts are

TABLE II
Chemical Shifts (ppm) of Chitosan Obtained by ^{13}C-NMR

Sample	C-1	C-2	C-3	C-4	C-5	C-6	CH_2	CH_3	C═O
Chitosan	105	58.0	75.7	83.7	75.7	58.0	—	24.7	173.6
QDAB	102	61.6	74.0	81.0	74.0	61.6	31.0	24.0	172.0
QAB	102	61.7	74.8	81.0	74.0	61.7	34.0	24.0	175.0

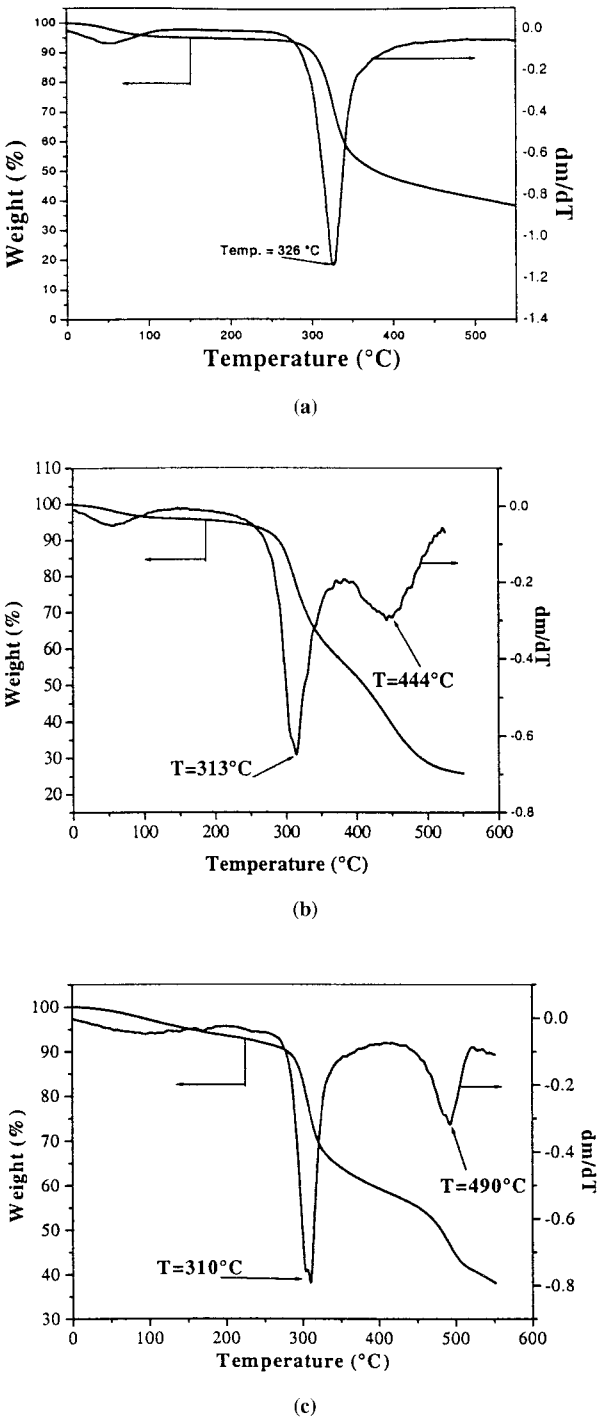


Figure 3 Thermogram and first derivative of (a) chitosan, (b) QDAB, and (c) QAB.

summarized in Table I. DA was determined as follows:

1. From the ratio of the relative intensities of the corresponding signals of the protons of —$COCH_3$ in the acetylated units divided by three and divided by the sum of the relative intensities of the signals of the protons on C-1:

$$\text{DA (\%)} = \left[\frac{(I_{CH3}/3)}{I_{H1+H1'}}\right] \times 100$$

2. From the ratio of the relative intensities of the corresponding signals of the protons of —CO—CH_3 in the acetylated units divided by three and divided by the sum of the areas of the signals of the protons on C-2:

$$\text{DA (\%)} = \left[\frac{(IA_{CH3}/3)}{I_{H2+H2'}}\right] \times 100$$

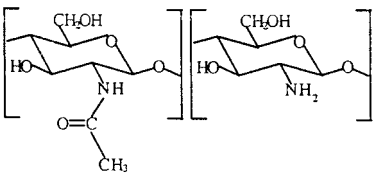
The average of the two methods gave DA = 3.5%.

FTIR spectroscopy

In Figure 1(a–c), the FTIR spectra of chitosan and its derivatives are shown. The FTIR spectra of the QDAB and QAB derivatives show the same chitosan band at 3440 cm^{-1} corresponding to ν_{OH}. This indicates that no substitution on this group occurs. The ratio between the relative intensity of the amide I (C═O) band at 1654 cm^{-1} and the band at 3400 cm^{-1} is greater in the derivatives than in chitosan. This may be considered an indication that free amine groups of chitosan reacted with the acid groups to produce an

TABLE III
Thermogravimetric Data of Chitosan and Its Derivatives

Sample	Temperature (°C)			Weight loss (%)
	Initial	Peak	Final	
First decomposition process				
Chitosan	250	326	440	51
QDAB	250	310	382	38
QAB	250	310	400	35
Second decomposition process				
QDAB	382	444	520	29
QAB	436	490	520	16



MW (g/mol):	203.19	161.15
Molar Fraction:	0.035	0.965
MW average:	162.62	

Scheme 1 Structure of chitosan.

amide bond. On the contrary, this intensity ratio in QAB is greater than in the QDAB derivative because the substitution obtained was higher.

The band at 1570 cm^{-1} in the spectrum of QDAB [Fig. 1(b)] is probably due to a combination of the N—H bending band (amide II) and the C═C stretching band of the substituted aromatic ring. These bands overlap the N—H bending of the free amine groups at 1596 cm^{-1} seen for chitosan. The band at 1470 cm^{-1} also corresponds to C═C conjugation in the substituted aromatic rings. A strong C—N stretching band that appears at 1424 cm^{-1} is typical of aromatic amines.

The band at 1559 cm^{-1} in the spectra of QAB is due to the N—H bending band and the C═C stretching of the substituting aromatic ring [Fig. 1(c)]. In QAB, this band has a lower intensity than in QDAB because the substitution in the aromatic ring is also lower. The $\nu_{C—N}$ peak in QAB spectra appears at 1380 cm^{-1}.

Solid ^{13}C-NMR spectroscopy

As can be seen in the ^{13}C-NMR spectrum [Fig. 2(a)], the CH_3 and C═O signals of chitosan have very low intensities because of the low DA of this polysaccharide. The signals corresponding to C2 and C6 appear as one broad signal. That is why it is not possible to differentiate among them.

In the ^{13}C-NMR spectra, QDAB shows the signals corresponding to the aromatic carbons around 120 ppm. The C═O signal at 172 ppm is higher than in chitosan, as shown in Figure 2(b). The great intensity of the bands corresponding to CH_2 at 31 ppm and CH_3 at 24 ppm is evidence of derivative formation.

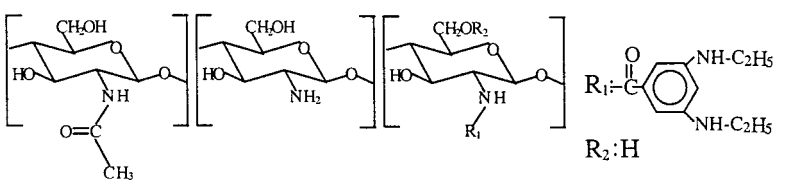
The solid ^{13}C-NMR spectrum of QAB is quite similar to that of QDAB. The signals of the aromatic carbons appear at 128 ppm. The C═O signal at 175 ppm is also higher than in chitosan [Fig. 2(c)]. The increase in the intensity of the corresponding signals of CH_2 at 34 ppm and of CH_3 at 24 ppm is also evidence of derivative formation. Table II shows the cross-polarity/magic-angle-spinning ^{13}C NMR data of chitosan and its derivatives.

Thermogravimetry study

A thermogram of chitosan is shown in Figure 3(a). This polysaccharide presented its main decomposition between 250 and 440°C, with a maximum weight loss at 326°C. In this process, 51% of the total mass was lost. The presence of a unique decomposition process is a typical behavior of homopolymers and in this case is indicative of the high deacetylation degree of chitosan. In Table III, the decomposition temperature and the weight loss associated with each decomposition process are summarized for chitosan and its derivatives.

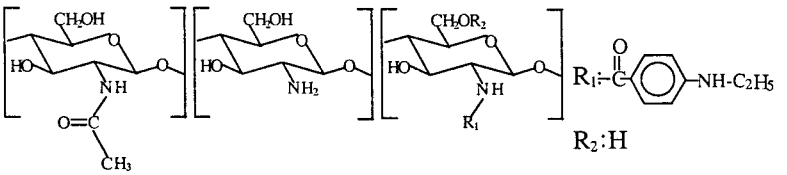
TGA of QDAB [Fig. 3(b)] shows a first peak due to water loss. At a higher temperature, two decomposition peaks can be seen. This behavior is typical of a block copolymer. In this case, each peak corresponds to substituted or unsubstituted glucosamine units. This block substitution pattern is probably due to the heterogeneous reaction media employed in its preparation. The substituted units are probably those near the surface, to which the reactant has access. The maximum decomposition rate of the second effect occurs at 313°C. This temperature is quite similar to that obtained for chitosan degradation and corresponds to the decomposition of the glucosamine unit. The other process is centered at 440°C and should be due to the decomposition of substituted units.

TGA of QAB [Fig. 3(c)] also shows two decomposition processes typical of block copolymers. The first process is centered at 310°C and should be due to the decomposition of the glucosamine units. This value is



MW (g/mol):	203.19	161.15	351.26
Molar Fraction:	0.035	0.671	0.294
MW average:		218.54	

Scheme 2 Structure of the QDAB derivative.



MW (g/mol):	203.19	161.15	308.24
Molar Fraction:	0.035	0.357	0.608
MW average:		252.05	

Scheme 3 Structure of the QAB derivative.

very similar to that obtained for chitosan and the QDAB derivative. The second effect has a maximum decomposition rate at 490°C. This process may be related to the decomposition of the substituted units. The aromatic moieties linked to the polymer backbone give the substituted units a thermal stability higher than that of pure chitosan.

Elemental analysis

The schemes show the most likely structures and molecular weights of the units of chitosan and its derivatives. The structural formula of chitosan was obtained with the DA calculated by ^{1}H-NMR (Scheme 1). The substitution degree of the derivatives was determined by elemental analyses.

From the elemental analyses, it was determined that QDAB (Scheme 2) was prepared with a substitution degree of 29% and QAB (Scheme 3) was prepared with a substitution degree of 60%.

CONCLUSIONS

The synthesis of QDAB and QAB was achieved. Degrees of substitution of 29% and more than 60%, respectively, were obtained. Both derivatives were characterized with solid-state ^{13}C-NMR, FTIR spectroscopy, and TGA. The presence of aromatic groups incorporated into chitosan increased the thermal stability of the substituted units.

These derivatives have potential as new products for the trapping of heavy metals, pigments, and proteins.

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