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The effect of Chitin Alkaline Deacetylation at Different Condition on Particle Properties

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Abstract

The abundant biopolymer chitin, found mainly in crustacean exoskeleton, such as crab, shrimp and lobster, can be deacetylated to yield chitosan. This slightly different biopolymer is more reactive than chitin, being more effective for many applications in fields as environmental remediation, biomedical sciences, catalysis and so on. The main process for chitin deacetylation used sodium hydroxide solutions at high temperatures for long times to obtain chitosan with high deacetylation degree (DD). The present study has evaluated the effect from room temperature (RT), 363 and 393 K, hydroxide concentration (2.0 or 10.0 mol dm⁻³) and time (3 and 24 h) on shrimp chitin deacetylation. Similar amounts of chitin and sodium hydroxide solutions were stirred jointly and the resultant solids were filtered and washed until pH 7, then dried at environmental conditions. The obtained samples were characterized by several techniques, such as elemental analysis, X-rays diffraction (XRD), laser scattering and absorption spectroscopy at infrared region with Fourier transform (FTIR), which was used for DD calculation. The results showed that all chitin-chitosan samples did not reach DD > 90 %, as observed for some good commercial chitosans. The highest DD was obtained by the sample prepared at more drastic conditions, as expected, however the higher sodium hydroxide concentration leads to decrease of molecular mass when associated with high temperatures. The crystallinity was influenced mostly by reaction time, which change the positions and intensities as indicated by XRD main peaks, located at 9.3 and 19.4 ° 2θ. Particle sizes were strongly diminished by treatment at 393 K, what imply also some increase at the pressure, favoring chain dissociation reactions. This work mapped several properties for chitin-chitosan samples achieved by the described conditions.

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1. Introduction

Chitin and chitosan are natural and abundant polymers with immense structural possibilities for chemical and physical modifications to engender novel properties, functions and applications, especially in environment and biomedical areas.¹ Despite its enormous availability, the use of chitin has been restricted by its intractability and insolubility. Thus, for many applications chitosan present better performance,²⁻⁴ due higher basicity at amino groups,³⁻⁶ which imply a correlation with the deacetylation degree (DD).⁷ Chitosan and chitin are linear copolymers composed by $\beta(1 \rightarrow 4)$ -[2-acetamido-2-deoxy- β -D-glucopyranose/2-amino-2-deoxy- β -D-glucopyranose] units⁸ as shown at Figure 1. Consequently, the amino/acetamido group ratio will define if the polymers are either as chitosan or chitin forms.⁹ Despite chitosan occurs as a component of the cell wall of some fungi, it is generally produced by carrying out the deacetylation of chitin, which is found in the shells of crustaceans, particularly lobsters, crabs and shrimps.¹⁰ Due to its particular physical, chemical and biological properties chitosan has widespread applications in the food industry,¹¹ pharmacy and medicine,^{12,13} agriculture,¹⁴ and wastewater treatment.^{6,15,16} Also many further modifications can be performed to improve and find out new uses in similar ways as observed for cellulose.¹⁷⁻¹⁹ Thus, this investigation consisted to determine the effect of chitin alkaline deacetylation at different conditions, regarding the DD achieved, morphology, particle charge and crystallinity. Since deacetylation process is already mastered by the industry, no study about these intermediate conditions were reported yet.

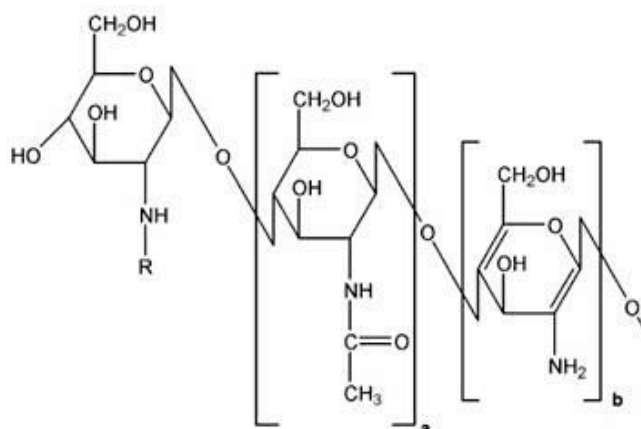


Fig. 1. Chitin/chitosan schematic basic units, 2-acetamido-2-deoxy- β -D-glucopyranose (a) and 2-amino-2-deoxy- β -D-glucopyranose (b).

2. Experimental Part

2.1. Sample preparation

Commercial sodium hydroxide, chitin and chitosan were purchased from Aldrich. The present study has evaluated the effect of temperature, hydroxide concentration and time on chitin from shrimp in the deacetylation process. Similar amounts of chitin and sodium hydroxide solutions were stirred jointly at room temperature or 363 K or even aged under static conditions at 393 K into a stainless steel autoclave, after previous mixing at room temperature. NaOH concentrations of 2.0 and 10.0 mol dm³ were employed while the reaction times were 3 and 24 h. The resultant solids were filtered and washed until pH 7, than dried at environmental conditions. The experimental conditions for each sample are summarized in Table 1 that also included some other results.

2.2. Characterization

The samples were analyzed by X-rays diffraction (XRD) on a diffractometer XRD 7000, Bruker, using a radiation source of $\text{CuK}\alpha$ 0.154 nm, with a scan rate of $2.0^\circ 2\theta \text{ min}^{-1}$ at room temperature. Relative crystallinity was calculated by the integration of the lowest angle peak and considering the highest value as 100 %. Some samples were analyzed by transmission electron microscopy (TEM). The samples were suspended in isopropyl alcohol, sonicated and deposited on a carbon thin film supported onto a copper grid. The micrographs were taken at the Electron Microscopy Laboratory (LME) of the National Synchrotron Light Laboratory (LNLS), the microscope was used JEM 2100 FEG with accelerating voltage of 200 KV. The zeta potential was determined by laser Doppler velocimetry using a NanoZetaS (Malvern Instruments LTD., UK). The ζ -potential was estimated from the electrophoretic mobility measurements using the Smoluchowski model. Elemental analysis was performed to determine the quantities of nitrogen, hydrogen and carbon using a Perkin Elmer model PE 2400 elemental analyzer. Infrared spectra of the samples in KBr pellets were obtained by accumulating 32 scans on a Bomem Spectrophotometer, MB-series, in the 4000 to 400 cm^{-1} range with resolution of 4 cm^{-1} . Deacetylation degree (DD) or the percentage of free amine groups ($-\text{NH}_2$) on the samples was determined based on the relationship between absorbance (A) values at 1655 and 3450 cm^{-1} by applying the equation $\text{DD} = 97.67 - [26.486(\text{A}_{1655}/\text{A}_{3450})]$.^{3,7,20}

3. Results and Discussion

Concerning some macroscopical issues it was observed that the temperature of 393 K and the higher hydroxide concentration has strongly modified color and the viscosity of the wet sample dispersion whilst and after filtering. Higher temperature darkened the samples and higher basicity increased the viscosity. On the other hand, the conditions did not lead to notable difference, however the higher reaction time lead to easier washing until pH 7, indicating a more complete interaction all along the sample with the basic groups.²¹

Table 1. Sample experimental conditions: temperature (T), time (t) and sodium hydroxide concentration ($[\text{OH}^-]$); percentages of the biopolymer elements, carbon/nitrogen ratio; infrared spectroscopy (FTIR) to determine the degree of deacetylation (DD); relative crystallinity (Cryst) by X-ray diffraction (XRD) and zeta potential (ζP).

Sample	Conditions			Elemental Analysis (%)				FTIR	XRD	ζP (mV)
	T (K)	t (h)	$[\text{OH}^-]$ (mol dm^{-3})	C	H	N	C/N	DD (%)	Cryst (%)	
Chitin	-	-	-	41.8	6.0	7.9	6.1	59.2	90	-14.3
C1	297	3	2	36.7	5.8	5.8	7.4	73.7	98	-7.6
C2	297	3	10	36.6	6.4	6.8	6.3	72.2	79	-7.2
C3	363	3	2	36.9	5.9	5.6	7.7	76.1	78	-4.0
C4	363	3	10	36.4	5.7	5.8	7.3	67.5	78	-3.1
C5	393	3	2	37.1	5.9	5.5	7.9	72.2	75	5.6
C6	393	3	10	28.9	5.1	4.8	7.0	75.1	72	-2.2
C7	297	24	2	39.6	5.7	5.8	8.0	71.2	100	-1.4
C8	297	24	10	40.5	6.1	6.2	7.6	72.3	87	1.5
C9	363	24	2	40.1	6.4	6.1	7.7	77.9	91	18.2
C10	363	24	10	38.3	6.2	5.8	7.7	76.4	69	22.8
C11	393	24	2	40.4	5.5	6.5	7.2	79.3	61	28.2
C12	393	24	10	34.6	5.1	5.0	8.0	84.4	36	8.3
Commercial chitosan	-	-	-	40.5	6.2	7.3	5.5	89.7	59	25.4

The chemical composition for all samples and one commercial chitosan, also from shrimp, were determined by elemental analysis and results are shown in Table 1. Due to possible further purification the commercial chitosan cannot be directly associated with the remains results. However, the C/N ratio for both chitin and chitosan commercial samples were lower than the theoretical expectations, 6 for chitosan and 8 for chitin. In general lines is possible to affirm that the time has stronger influence on the final C/N ratio, leading to higher number at 24 h, while the other components do not directly influenced.

The X-rays diffraction patterns for all prepared sample are shown at Figure 2A. A typical diffractogram for chitin, with all regular peaks was obtained in curve a. Subsequent modification leads to slight changes in most cases, presenting only differences regarding the intensities. Only the sample C12, prepared with higher temperature, reaction time and hydroxide concentration, has shown some significant peak displacement to higher angles. This change and also the peak broadening are due a strong morphological modification with some structural contribution. The regular fibrous structure changes for a polyhedral nanoparticle system, as confirmed later by TEM image. The calculated values for relative crystallinity are shown in Table 1 and in this case really mean the level of organization, but not exactly the absolute crystallinity, since these biomaterials did not behave as real crystals.²² It was noticed that chitin does not present the highest crystallinity value, but C7 and C1, which were submitted to mild condition with probably dissolved part of the amorphous portion of chitin. However, as expected, more drastic conditions lead to a decrease on the polymer organization, influenced mainly by the time and the temperature, to give to C12 sample only 36 %, even lower than observed for a commercial chitosan.

The chitin/chitosan infrared absorption spectra are shown in Figure 2B. The samples present characteristic bands, such as C-H stretchings, located around 2920 and 2850 cm^{-1} , for O-H and N-H stretchings, which are overlapped and centered around 3400 cm^{-1} , the band at 1320 cm^{-1} is due to aliphatic CH bending vibrations. The chitin presence is associated with acetamide group, which presents its bands at 1655 and 1380 cm^{-1} , attributed to C=O and C-H deformation bands, respectively.⁶ Bands between 1250 and 800 cm^{-1} are related with the pyranosidic ring, reflecting C-O-C and **b glycosidic** linkages and also C-O bond from primary and secondary alcohols. Thus, the main dissimilarity among those spectra is concerning the relative transmittance of acetamide groups, and results are translated into DD values, which are shown in Table 1.

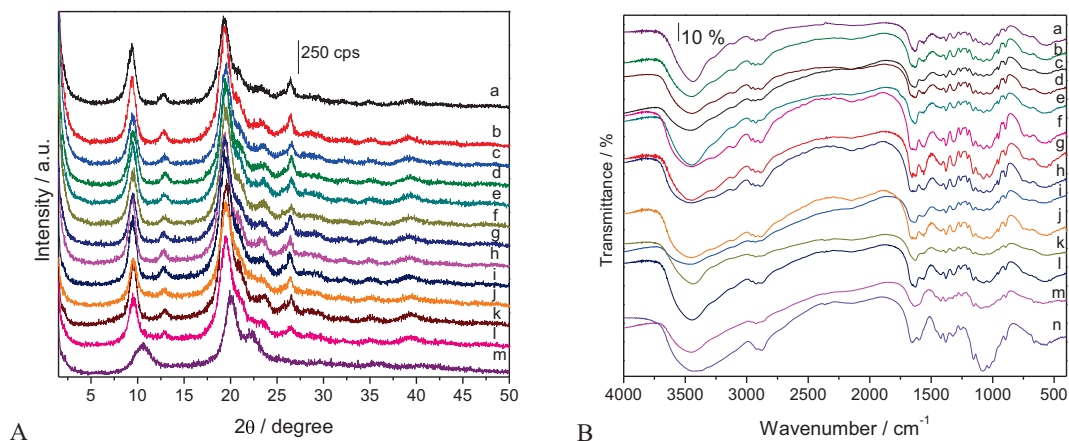


Fig. 2. XRD patterns (A) and FTIR spectra (B) for the samples: chitin (a), C1 (b), C2 (c), C3 (d), C4 (e), C5 (f), C6 (g), C7 (h), C8 (i), C9 (j), C10 (k), C11 (l), C12 (m) and chitosan (n).

Despite some authors name chitosan the biopolymer with DD > 50%,^{23,24} other researchers do not use such a straight line to define if the material is chitin or chitosan.²⁵⁻²⁷ Nevertheless, the chitin sample present a DD value of 59.2 % and can be used to compare other samples DD values. With exception of C4 sample, all obtained materials have DD higher than 70 %. In general, it could be saying that the reaction time is very important when associated with higher temperatures, while at room temperature DD kept the same or slightly smaller. Most samples possess

DD ranging from 71 to 79 % and the exception with higher value was C12, prepared at more drastic condition, to give a DD value of 84.4 %. This value is very near to that obtained by the commercial chitosan, 89.7 %, indicating the good efficiency for this process in this established condition.

Particle charges were measured by the zeta potential, and the obtained values are shown in Table 1. Roughly, it was observed that chitin and most samples prepared with only 3 h (C1-C4, C6) present negative charge, while chitosan and the samples submitted to longer reaction times (C8-C12) are positively charged. Also in spite of C12 present higher DD and lower crystallinity, its charge was considerably smaller than chitosans and C9-C11 showed more similar results.

The morphological characterization of some samples was performed by TEM and images for C12 sample are shown in Figure 3. The sample present not so homogeneous particle shape distribution and diverse regular or not polyhedral particles gave 10-37 nm in two or three dimensions. Agglomerations of many particles took places from few to many hundreds of nanometer, having 417 nm as an average. Despite XRD shows clear diffraction peaks in the microscope, no clear diffraction spots were observed, which are also due beam damage and agree with theoretical expectations.²⁸ This strong change on particle size and organization degree can be correlated with the lower zeta potential that can be stabilized by the formation of agglomerates.

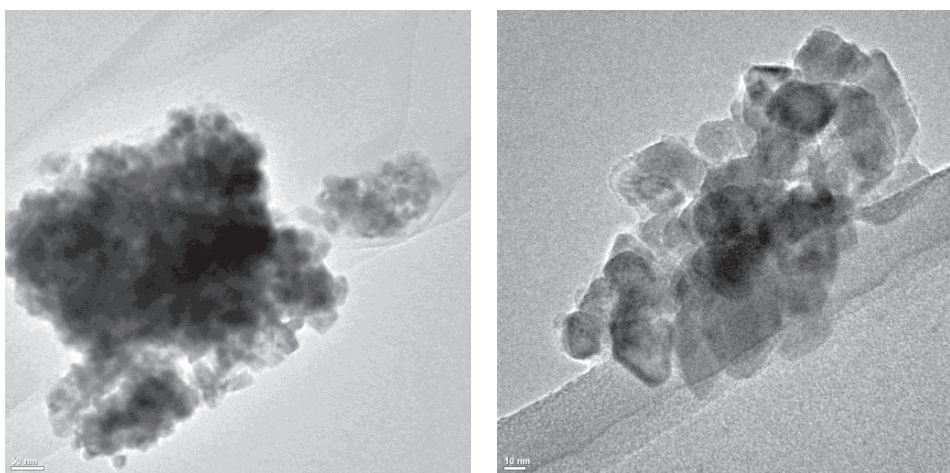


Fig. 3. TEM images of C12 at different magnifications.

4. Conclusions

The set of results have mapped alkaline chitin deacetylation effects on some properties. Reaction time had stronger influence to change most of these properties, mainly when allied with higher temperature, 363 or 393 K, while at room temperature the conversion of chitin into chitosan reach its maximum quicker and no significant differences regarding the time, were noticed. Sodium hydroxide concentration demonstrated the slightest influence on this process, except for the samples prepared with 24 h and at 393 K. In this case the higher hydroxide concentration lead to a chitosan sample, C12, completely distinct from all other, with higher DD values, weaker charged and with a very different morphology, consisting on nanoparticles always smaller than 50 nm. These properties allow this sample to be used in many distinguished application that need smaller particle sizes.^{29,30} In spite this work has determined the sample properties for those experimental conditions, further studies should be carried out to investigate the role of each experimental setting to elucidate the reaction mechanism in details.

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