



HAL
open science

Influence of chitosan characteristics on polymer properties. I: Crystallographic properties

Malgorzata Jaworska, Kensuke Sakurai, Pierre Gaudon, Guibal Eric

► To cite this version:

Malgorzata Jaworska, Kensuke Sakurai, Pierre Gaudon, Guibal Eric. Influence of chitosan characteristics on polymer properties. I: Crystallographic properties. *Polymer international*, 2003, 52 (2), pp.198-205. <10.1002/pi.1159>. <hal-04664071>

HAL Id: hal-04664071

<https://imt-mines-ales.hal.science/hal-04664071v1>

Submitted on 17 Mar 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Influence of chitosan characteristics on polymer properties. I: Crystallographic properties

Malgorzata Jaworska,¹ Kensuke Sakurai,² Pierre Gaudon³ and Eric Guibal^{4*}

¹Warsaw University of Technology, Faculty of Chemical Processes and Engineering, ul Waryńskiego 1, 00-645 Warsaw, Poland

²Fukui University, Faculty of Engineering, Department of Materials Science and Engineering, Bunkyo, Fukui 910-8507, Japan

³Ecole des Mines d'Alès, Centre des Matériaux de Grande Diffusion, 6, avenue de Clavières, F-30319 Ales cedex, France

⁴Ecole des Mines d'Alès, Laboratoire Génie de l'Environnement Industriel, 6, avenue de Clavières, F-30319 Ales cedex, France

Abstract: The properties of chitosan depend on several parameters including its origin (shrimp, squid, fungi etc), characteristics (mol fraction of *N*-acetylation; molecular weight) and treatments used to condition it (dissolving, precipitation, drying). These parameters can influence the material's sorption properties for metal ions. It has been suggested that the changes in sorption properties are related to the crystallinity of the material. The present work focuses on the study of the crystallographic properties of samples prepared from shrimp, squid and fungi sources, with different characteristics and conditioned by several physical treatments (dissolving, reprecipitation and drying, including oven-drying, freeze-drying), prior to subsequent studies of their sorption properties for platinum (discussed in Part II of this manuscript).

Keywords: chitosan; fraction of acetylation; chitin origin; crystallography; dissolving; drying

INTRODUCTION

Chitosan is a natural and biodegradable biopolymer produced from chitin, the most abundant biopolymer after cellulose, by means of an alkaline deacetylation procedure. It is characterized by properties that are unique among natural polymers and is a cationic polymer in acidic solutions. It has been extensively studied for applications in areas as diverse as wastewater treatment (flocculating properties, chelation and ion exchange properties), cosmetics (hair and skin care), paper and textiles (conditioning polymer), biomedicine and biology (drug delivery, enzyme immobilization), and agro-industries (seed treatment). Since chitosan is a heterogeneous polymer consisting of D-glucosamine and *N*-acetyl-D-glucosamine units, its properties depend on its structure and composition. In the abundant literature on chitosan applications it is generally difficult to find reproducible information on process performance since the characteristics of commercial chitosan materials are highly variable with regard to the molfraction of *N*-acetylation (F_A), molecular weight (MW) and crystallinity. For example, in the case of metal ion sorption, it is difficult to compare sorption properties not only because of the different experimental conditions selected by researchers, but also due to the lack of information on the characteristics of the samples used. The

discrepancies observed between the different studies may be related to differences in the characteristics of the chitosan.

These differences may be explained by differences in the origin of the material.¹ Chitin can be produced from materials as different as fungal biomass,²⁻⁴ crustacean shells⁵ and insect cuticles.⁶ Depending on the source of the material, several forms of chitin can be obtained: these different types are defined in relation to the packing of the polymer chains, whether in an anti-parallel arrangement (α -chitin), parallel arrangement (β -chitin) or mixed arrangement (two chains above for each chain below) as in γ -chitin. The most stable form (and the most abundant) is α -chitin, which usually appears in the cuticle of arthropods (associated with sclerotized proteins and the inorganic part of the cuticle). The other forms (β -chitin and γ -chitin), which are easily transformed into α -chitin, are found, for example, in the skeletal pen and stomach cuticle of squid, giving the material a flexibility and toughness that contrasts with the hardness of materials made of α -chitin (crustacean shells).⁷ In addition, the origin may affect the residual crystallinity of chitosan, which in turn controls the accessibility to internal sorption sites and the diffusion properties (re-hydration and solute transport). Kurita *et al* also observed a greater reactivity of β -chitin

compared with α -chitin for the preparation of chemical derivatives.⁸

Another reason for possible variations in the sorption properties of the material is the change in the structure of the polymer as the result of physico-chemical modifications. For example, the *N*-deacetylation procedure may affect the solid state structure of chitosan.⁹ The choice between the homogeneous or heterogeneous procedure may also strongly influence the characteristics of the polymer. Ogawa and Yui studied the crystallinity of different chitin/chitosan samples prepared by two different procedures: (a) the partial deacetylation of chitin, and (b) the partial re-acetylation of a fully deacetylated chitin (pure chitosan).¹⁰ They observed that the partially re-acetylated material was less crystalline than pure chitosan. They also showed that, for the preparation of a less crystalline chitosan sample (partially acetylated at a given F_A), it is preferable to proceed via re-acetylation of fully deacetylated chitosan rather than direct solid-state deacetylation of chitin. This preferred treatment also resulted in less anhydrous crystals.

The conditioning of the polymer also influences sorption properties: the preparation of chitosan gel beads proved an interesting way to improve the diffusion properties of the polymer.¹¹ This enhancement may be linked to both an expansion of the polymer network and a decrease in the residual crystallinity of the material. Generally, the step of dissolving the polymer results in a decrease in the crystallinity of the material. However, it also depends on the secondary treatment (reprecipitation and drying, freeze-drying).

This work focuses on the comparison of platinum sorption performances at pH 2 for different chitosan samples after glutaraldehyde cross-linking. The sorption performances (kinetics and equilibrium) will be compared for the different materials (Part II).¹² This first part focuses on the crystallographic characterization of the different polymers, using X-ray diffraction analysis. Samples produced from fungal, shrimp and squid chitin were tested. The F_A was varied and, for some of these samples, the polymer was re-conditioned with a combination of different treatments including dissolving, precipitation and drying (oven-drying, freeze-drying).

MATERIALS AND METHODS

Materials

France Chitine (France) kindly donated pure squid and shrimp chitin samples. The deacetylation of chitin was performed by contact of grinded chitin samples with NaOH solution (20%, w/w) at 121 °C in an autoclave for contact time ranging between 10 and 30 minutes, depending on the degree of deacetylation to be reached. Chitosan of fungal origin was obtained from cultures of *Absidia orchidis* (NCAIM F 00642) on selected media: chitosan was obtained by a two-step

procedure: alkaline deproteination and acid extraction.³ When required, chitosan samples were dissolved in acetic acid solution (1%, w/w) at a concentration of 0.5% (w/w). The solution was left to stand overnight and then the biopolymer was re-precipitated with sodium hydroxide solution (1 M). Some samples were also oven-dried or freeze-dried directly from solutions. The gel phase was washed out till the pH remained constant (neutral) and was then collected by centrifugation (6000 rev min⁻¹ for 20 min). The solid was oven-dried or freeze-dried. The oven-drying was performed at 60 °C overnight. When the samples were freeze-dried, the drying was carried out at -24 °C.

Analysis procedures

The degree of deacetylation was determined using a FTIR spectrometric method.¹³ Sorbents were cross-linked with glutaraldehyde (GA) with a fixed molar ratio between aldehyde functions and -NH₂ groups (1 mol of GA for 1 mol of -NH₂). The chitosan was wetted for 1 h in demineralized water, the appropriate amount of GA was then added and the mixture was agitated overnight. Finally the solid was rinsed several times with water (10 times the volume used for cross-linking). The diameter of sorbent particles was less than 125–250 μ m.

The X-ray diffraction patterns were determined using a wide-angle X-ray Philips PW1729 diffractometer and the $K_{\alpha, \text{Cu}}$ radiation. The voltage was 40 kV and the intensity 40 mA. The 2θ angle was scanned between 3° and 60°, and the counting time was 1.2 s at each angle step (0.05°). In the figures, the X-ray diffractograms (XRD) are presented only for the 2θ angle range between 3° and 30°. The distance between the plans in each direction was calculated using the Bragg equation (the wavelength of the $K_{\alpha, \text{Cu}}$ radiation is 1.5405 Å). The full width at half the maximum (FWHM) was measured after correction by subtracting the baseline for the amorphous region of the XRD patterns in order to obtain a measurement of the size of the crystals of the sample. The FWHM due to the equipment (source diffraction) was measured on quartz powder and was systematically subtracted from the experimental values. The Scherrer equation relates the FWHM ($\theta(2\theta)$) to the size of the crystallites (D), the wavelength of the radiation (λ , for $K_{\alpha, \text{Cu}}$ radiation it is 1.5405 Å), and to the peak angle (θ , in rad):

$$\Delta(2\theta) = 0.9\lambda / (D \cos \theta)$$

Since the peak at $2\theta = 20^\circ$ is asymmetrical on most of the XRD patterns (superimposition of two peaks), the same calculations were also performed considering the left part of the peak and doubling its width to obtain the FWHM for the left part (FWHM_l), instead of the FWHM for the whole peak (FWHM_w). The ratio FWHM_w/FWHM_l was used to calculate an asymmetrical coefficient for the peak $2\theta = 20^\circ$. The Scherrer equation was used with both FWHM_w and FWHM_l to calculate the size of the crystallites (taking into account

the complete width of the peak, D_w , or only the part corresponding to left peak, D_l).

The total area of the XRD patterns divided by the area of the crystalline regions (delimited from the upper part of the XRD patterns after correction of the baseline for amorphous regions, and after correction of the general baseline) gave the crystalline index (Cr I_{area}).

Another technique for calculating the crystallinity index was also used. Based on the method proposed by Focher *et al.*,¹⁴ it consisted in measuring the maximum intensity, I_{110} , at $2\theta=20^\circ$ of the (110) lattice diffraction and that of the amorphous diffraction, I_{am} , at $2\theta=16^\circ$.¹⁴ The crystallinity index was calculated using the following formula:

$$Cr I_{peak} = (I_{110} - I_{am})/I_{110}$$

These parameters were determined on samples before cross-linking. Tual *et al* have shown that heterogeneous cross-linking does not affect the crystallinity of the chitosan.¹⁵ In the case of homogeneous modification, Kurita *et al* showed that cross-linking of chitosan with GA decreases chitosan crystallinity, at low degree of substitution.¹⁶ Monteiro and Airolidi (1999) reached a similar conclusion for the homogeneous cross-linking of chitosan.¹⁷ In the present study the cross-linking was carried out under heterogeneous conditions and we assume the crystallinity not to be influenced by the cross-linking treatment.

RESULTS AND DISCUSSION

It is interesting to note from Figs 1–4 that, except in the case of chitin samples, the XRD were characterized by two scattering angles at $2\theta \approx 10^\circ$ and $2\theta \approx 20^\circ$. The third peak that usually appears at $2\theta \approx 23^\circ$ for the allomorphic tendon form of chitosan was not present, only very small shoulders appeared for some of the samples.¹⁸ Piron also observed a decrease in this peak for a sample which had been dissolved, and its disappearance for a freeze-dried sample. The lack of this peak may indicate that the commercial samples had been submitted to some dissolving during the preparation procedure.¹⁹

The crystalline index calculated by the area method (Cr I_{area}) gave significantly lower indices than the method based on the intensity of the signals (Cr I_{peak}) for the crystalline and amorphous regions ($2\theta \approx 20^\circ$ and $2\theta \approx 16^\circ$, respectively). Though the general trend was respected, it was not possible to positively correlate the data, as shown by Table 1.

Influence of the origin of chitin/chitosan on the crystallinity

The XRD patterns were characterized by broad peaks around $2\theta=9-10^\circ$ and $2\theta=19-20^\circ$ (this latter peak was asymmetrical). The XR diffractograms were significantly changed at $F_A: 0.1$ and $F_A: 0.5$: the intensity of the signals increased in the order fungi < shrimp < squid (Fig 1). The comparison of the XRD patterns

for $F_A: 0.1$ and $F_A: 0.5$ showed that the intensity increased with the F_A . The less deacetylated the sample, the higher its crystallinity. The least crystalline material was produced from fungal biomass and the most crystalline was produced from squid material. Obviously the peak at 10° was significantly smaller for the fungal source than for the two other materials.

In the case of chitin samples, there were large differences in the XRD of chitin from squid and shrimp origin. The XRD was significantly more resolved in the case of shrimp material than for squid material. Kim *et al*²⁰ and Li *et al*²¹ obtained similar XRD for α -chitin and β -chitin, respectively. While for squid material the diffraction pattern was comparable to that of deacetylated products with a peak at $2\theta=8.05^\circ$ (intersheet distance: 10.97 Å, this peak was significantly shifted) and $2\theta=19.6^\circ$ (4.52 Å), in the case of shrimp material, six signals were identified: at $2\theta=9.2^\circ$ (9.6 Å), $2\theta=12.55^\circ$ (7.05 Å), $2\theta=19.15^\circ$, $2\theta=20.8^\circ$ (a shoulder), $2\theta=22.95^\circ$ (3.87 Å) and $2\theta=26.15^\circ$ (3.40 Å). For squid material, the peaks that appeared between $2\theta=19^\circ$ and $2\theta=24^\circ$ for shrimp chitin disappeared and were replaced by a broad peak (representing the convolution of different reflections). Table 1 shows the crystalline indices calculated by both area and peaks methods. The

Table 1. Crystallinity index (Cr I) calculated by the area method (Cr I_{area}) and the peaks method (Cr I_{peaks}) of chitin and chitosan samples in function of the origin of the material, the molfraction of *N*-acetylation and the physico-chemical treatment (dissolving, precipitation, drying)

Origin and F_A	Treatment	Cr I_{area} (%)	Cr I_{peaks} (%)
Sh-Chitin	—	68.2	89.9
Sh-0.50	—	43.5	66.2
Sh-0.50	DD	41.0	69.6
Sh-0.50	DF	42.1	64.3
Sh-0.40	—	52.4	66.8
Sh-0.40	DD	36.2	47.2
Sh-0.40	DPD	59.3	71.2
Sh-0.40	DPF	50.8	70.5
Sh-0.37	—	48.4	74.0
Sh-0.37	DD	41.6	73.2
Sh-0.37	DF	36.2	60.9
Sh-0.19	—	42.0	70.9
Sh-0.19	DD	40.4	70.9
Sh-0.19	DF	35.2	56.7
Sh-0.10	—	42.4	67.6
Sq-Chitin	—	54.3	82.6
Sq-0.25	—	36.5	67.0
Sq-0.17	—	39.5	67.5
Sq-0.10	—	38.3	72.8
F-0.50	*	39.3	71.6
F-0.10	*	36.5	63.1

—, raw material; DD, dissolved dried sample; DF, dissolved freeze-dried sample; DPD, dissolved precipitated dried sample; DPF, dissolved precipitated freeze-dried sample.

* The method of chitosan production from fungi includes a step of chitosan dissolving (extraction of chitosan from 'cell walls' with acetic acid) followed by a precipitation step with NaOH and a final drying (equivalent to DPD treatment).

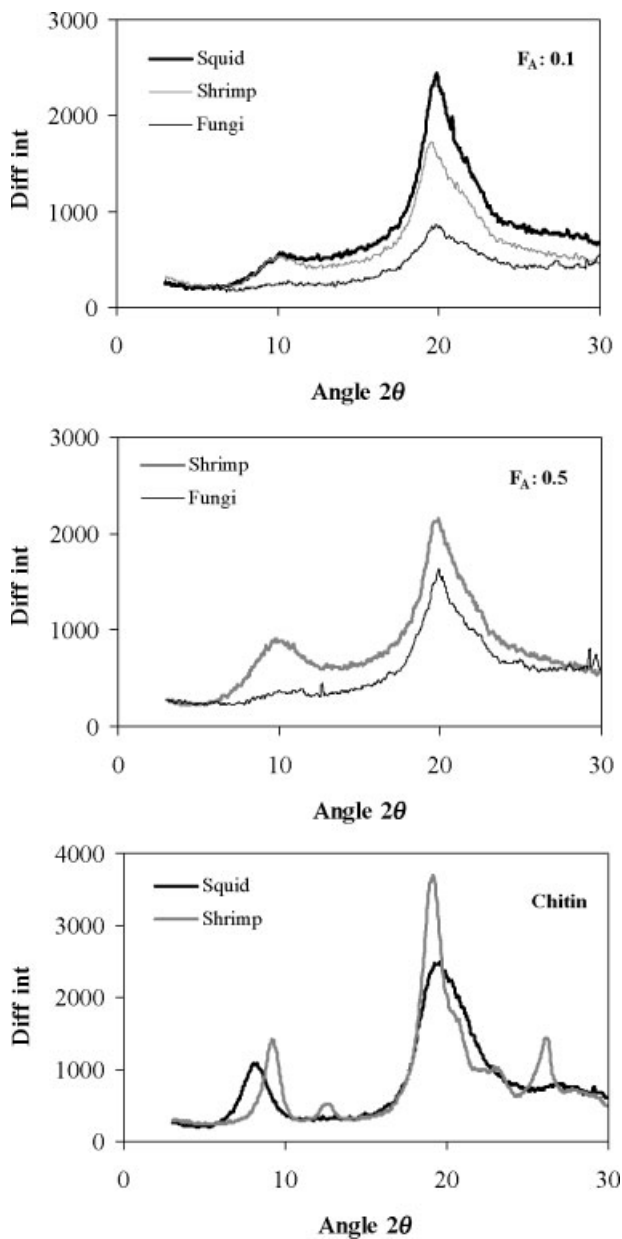


Figure 1. Influence of chitosan origin on X-ray diffraction patterns at different F_A . Bottom panel: XRD patterns for chitin.

crystalline indices were significantly higher for shrimp chitin than for shrimp chitosan. For partially deacetylated materials (F_A : 0.10), the crystalline indices were lower for fungal chitosan than for shrimp and squid chitosan. Similar trends were observed for the crystallinity index: that of shrimp chitin was significantly higher than that of squid chitin. Regarding chitosan samples, independently of the F_A , the crystallinity index increased in the following order: fungal chitosan < squid chitosan < shrimp chitosan.

The intersheet distance is influenced by the hydration of the material (from 9.3 Å to 11.1 Å, or 10.97 Å in the present case): the higher the hydration the higher the intersheet distance along the c axis.⁷ The origin of the material strongly affected its crystallinity, depending on the kind of chain arrangement as discussed in the introduction. Moreover, Roberts⁷ has explained

that in α -chitin the intersheet hydrogen bonds between adjacent chains (along the c axis) are responsible for the polymer not swelling in water, whereas β -chitin, in which intersheet bonding is absent, swells readily in water and forms hydrate. This property may influence the efficiency of the deacetylation procedure and the diffusion of water and metal ions in the ‘porous-structure’ of the biopolymer. This observation contrasts with the results of this study, and no explanation was found for this unexpected trend. Saito *et al* studied the swelling characteristics of β -chitin and their influence on its conversion into α -chitin.^{22,23} They observed three different levels of swelling depending on the experimental conditions: under mild conditions the structure was maintained despite the inter-sheet swelling. Under more acidic conditions, inter-sheet and intra-sheet swelling occurred simultaneously, while under the harshest acidic conditions, *ca* in HCl, 7–8 M, complete inter-sheet and intra-sheet swelling occurred and the structure lost its parallel organization to form α -chitin crystals with antiparallel chains.

Table 2 shows the FWHMs and the sizes of the crystals corresponding to the angle $2\theta \approx 20^\circ$, using the two calculation procedures. The order of magnitude was consistent with previous data from Piron.¹⁹ Other peaks were less resolved and the determination of the peak angles, FWHM was made difficult because of this low resolution. However, the data presented in the table are only indicative of the order of magnitude; indeed, the difficulty in determining the amorphous region on the XRD made these calculations approximate. For chitin samples, there was a great change in the size of the crystallites between materials produced from shrimp and squid sources: the size of the crystals was halved for squid material compared with shrimp chitin. For chitosan samples with F_A : 0.10, small differences were observed: the size of the crystallites (D_w) was between 2.7 and 3 nm. The differences were much more marked using the left part of the peak at $2\theta \approx 20^\circ$ (D_1): the squid material exhibited large crystal size, which is a reverse trend compared to with chitin material. For F_A : 0.50, shrimp and fungal chitosan were characterized by a similar size of crystals (D_w) at 3.3 nm, though the size of the crystals calculated by the second method (D_1) were significantly different. The correction of the FWHM, taking into account the asymmetry of the peak, strongly affected the determination of the size of the crystals.

Influence of the fraction of acetylation on the crystallinity

Figure 2 compares the XRD for chitosan produced from squid and shrimp, at different F_A . The XRD for corresponding samples was also presented in order to compare deacetylated products with the original material. The most significant differences appeared on the XRD of chitin samples while the differences were not so marked for deacetylated products. The chitin was much more crystalline than deacetylated material particularly in the case of shrimp material.

Origin and F_A	Treatment	$FWHM_w(2\theta)$	$FWHM_l$	Asym coeff	D_w (nm)	D_l (nm)
Sh-Chitin	—	1.47 (19.2)	1.46	1.01	5.7	5.8
Sh-0.50	—	2.58 (19.7)	1.91	1.35	3.3	4.4
Sh-0.50	DD	2.52 (20.0)	2.16	1.17	3.4	3.9
Sh-0.50	DF	2.52 (19.8)	1.97	1.28	3.4	4.3
Sh-0.40	—	3.38 (19.8)	2.67	1.27	2.5	3.1
Sh-0.40	DD	7.93 (20.0)	4.95	1.59	1.1	1.7
Sh-0.40	DPD	3.05 (20.0)	2.62	1.16	2.8	3.2
Sh-0.40	DPF	2.65 (19.8)	1.96	1.35	3.2	4.3
Sh-0.37	—	2.24 (19.6)	1.59	1.41	3.8	5.3
Sh-0.37	DD	2.57 (20.0)	2.05	1.25	3.3	4.1
Sh-0.37	DF	2.65 (19.8)	2.46	1.08	3.2	3.4
Sh-0.19	—	2.02 (19.6)	1.43	1.41	4.2	5.9
Sh-0.19	DD	2.52 (20.1)	2.24	1.13	3.4	3.8
Sh-0.19	DF	2.79 (20.0)	2.78	1.01	3.0	3.0
Sh-0.10	—	3.13 (19.6)	1.96	1.60	2.7	4.3
Sq-Chitin	—	3.27 (19.6)	2.35	1.39	2.6	3.6
Sq-0.25	—	2.92 (20.0)	2.51	1.16	2.9	3.4
Sq-0.17	—	2.79 (20.1)	2.51	1.11	3.0	3.4
Sq-0.10	—	2.85 (20.1)	1.39	2.05	3.0	6.1
F-0.50	DPD	2.57 (19.9)	1.39	1.85	3.3	6.1
F-0.10	DPD	2.99 (19.9)	2.24	1.33	2.8	3.8

Table 2. FWHM and crystallite size (D) of chitin and chitosan samples in function of the origin of the material, the molfraction of N -acetylation and the physico-chemical treatment (dissolving, precipitation, drying)^a

—; raw material; DD, dissolved dried sample; DF, dissolved freeze-dried sample; DPD, dissolved precipitated dried sample; DPF, dissolved precipitated freeze-dried sample.

^a FWHM and crystallite size are calculated on the peak at $2\theta = 20^\circ$, for the left part (1) and the complete width (w) of the peak. The asymmetrical coefficient (Assym coeff) was also calculated from these data.

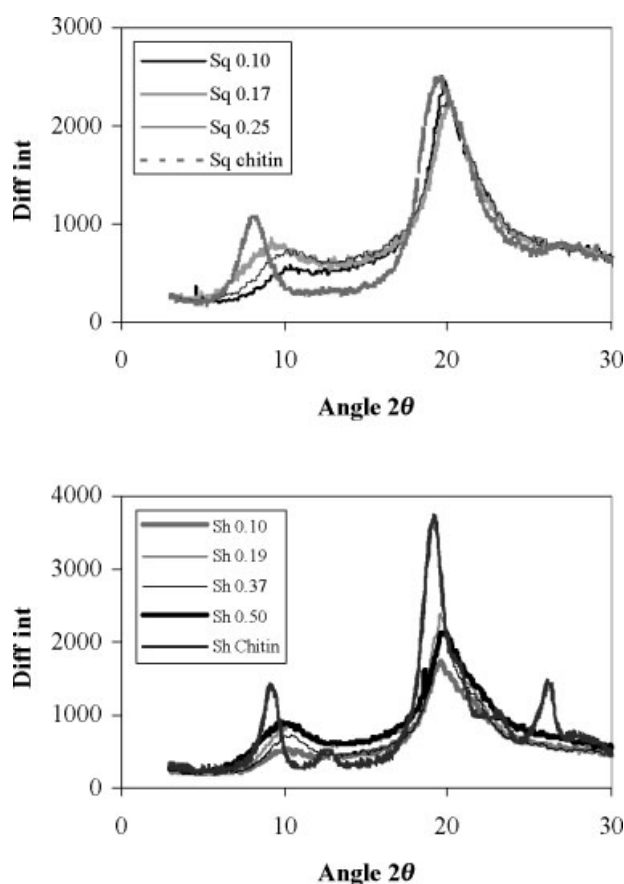


Figure 2. Influence of F_A on the X-ray diffraction patterns of chitosan from squid (top panel) and shrimp (bottom panel).

For this material, the deacetylation procedure involved the disappearance of many peaks: only two peaks were maintained at $2\theta = 9-10^\circ$ and $2\theta = 19-20^\circ$. Even for low deacetylation extent (high F_A) the modification of the biopolymer decreased the crystallinity of chitosan. This is consistent with results from Li *et al*, who observed that increasing the contact time of α -chitin with sodium hydroxide solution (at $95-97^\circ\text{C}$) resulted in a decrease in the crystallinity index of the material.²¹ Okuyama *et al* identified and characterized several XRD for chitosan depending on the arrangement and packing of polymer chains: the so-called Type I and Type II forms.^{24,25} Type I corresponds to the twofold helical structure with about 10\AA axial repeat (following the fibre c axis), while the Type II corresponds to a relaxed version of the twofold helix, ie ‘eightfold’ ($8/3$ or $8/5$) helical structure with 40\AA axial repeat.²⁵ The structure of chitosan (Type I or Type II speciation) strongly depends on the treatment of chitosan material: complexation with metal ions or contact with selected acids (organic or minerals). Preparing chitosan membranes, Urbanczyk and Lipp-Symonowicz observed that the type of crystalline structure, the degree of crystallinity and the average lateral crystallite size depend on the deacetylation degree of the material: in the case of higher F_A , the degree of crystallinity and the size of the crystallites were larger than those obtained from low F_A samples.²⁶ This is consistent with the results of the present study.

In the case of shrimp chitosan, the crystallinity index calculated by the area method varied between 42 and

48% for the different samples, significantly lower than the crystallinity index of shrimp chitin (68%). No continuous tendency was found: a maximum value was found at F_A : 0.40. When using the other procedure for the calculation of the crystalline index, the values varied between 66% and 74%; in this case the maximum crystallinity was obtained with F_A : 0.37. For squid chitosan, the index of crystallinity, $Cr I_{area}$, was almost constant between 36 and 40% (between 67 and 73% using the $Cr I_{peak}$), for F_A ranging between 0.10 and 0.25. In this case the crystallinity index was also significantly higher for squid chitin at 54% ($Cr I_{peak}$: 83%).

Fungal chitosan was characterized by crystallinity indices comparable to those of squid chitosan. The size of the crystallites was also influenced by the F_A . The maximum crystal size was found at F_A : 0.19 for shrimp chitosan (D_w : 4.2 nm instead of 2.7–3.8 nm for the other samples; D_1 : 5.9 nm instead of 3.1–5.3 nm). The asymmetric coefficient tended to increase with decreasing F_A . In contrast, for fungal and squid chitosan the size of the crystals was hardly changed by changing the F_A . While in shrimp chitin the size of the crystals was significantly higher than that of the chitosan samples, in the case of squid material the crystal size (D_w) slightly decreased compared with the deacetylated polymer. However, the D_1 was significantly higher for F_A : 0.10 than for the other samples.

Influence of the dissolving and drying procedure on the crystallinity

Salmon and Hudson reported chitosan unit cell parameters from published literature and illustrate the differences in these parameters with the treatment and conditioning of the polymer (physical state, annealing, etc).²⁷ Yui *et al*²⁸ and Okuyama *et al*²⁴ established the structural and crystalline characteristics of chitosan in anhydrous and hydrated forms, respectively. Significant differences were observed as an additional confirmation of the influence of the drying treatment on the crystallinity of the samples. Figure 3 shows the influence of the dissolving and drying (oven-drying or freeze-drying) process on XRD for shrimp chitosan at different F_A . The shape of the XRD of raw material (R) was maintained but the intensities of the peaks at $2\theta=9-10^\circ$ and $2\theta=19^\circ$ were significantly reduced after the dissolving/drying procedure. The intensity decreased more when the sorbent was dried by the freeze-drying procedure, this treatment reduces the crystallinity of the material. This is consistent with previous results from Piron.¹⁹

Ogawa compared the influence of heating an aqueous suspension of chitosan on its crystallinity for several chitosan samples characterized by different F_A and observed that chitosan with a low F_A was highly crystallized by heating and the XRD was characterized by the appearance of a peak at $2\theta=15^\circ$ at high temperature, especially for the most deacetylated sample.¹⁸

In our study, the decrease in the intensity was

stronger for low F_A than for high F_A in the case of the peak $2\theta=9-10^\circ$ while for the peak at $2\theta=19^\circ$ the decrease in the intensity was especially sensitive for high F_A . Dissolving chitosan led to a decrease in the crystallinity of the material, but the oven-drying of the material resulted in a more crystalline material than with freeze-drying. It is possible that the oven-drying partially restored the crystallinity of the biopolymer. Roberts has compiled the results for crystal structures (unit cell dimensions) from different research groups obtained through different processes.⁷ The results of different authors were highly divergent according to the technique they used for conditioning the material: simple deacetylation, re-precipitation, film-casting or annealing.^{18,23,27-31}

Dissolving the chitosan, especially in the case of low F_A , significantly modified the crystallinity index (both $Cr I_{peak}$ and $Cr I_{area}$). It followed a reciprocal trend to that observed for the intensity of XRD. The dissolving and drying operations did not change the size of the crystals at high F_A , but significantly decreased their size at low F_A . However, the type of drying procedure hardly influenced the size of the crystals, D_w . In most cases, the asymmetric coefficient of the peak at $2\theta=20^\circ$ decreased after dissolving and drying. As a consequence, the variation in the D_1 was much more marked with the different treatments.

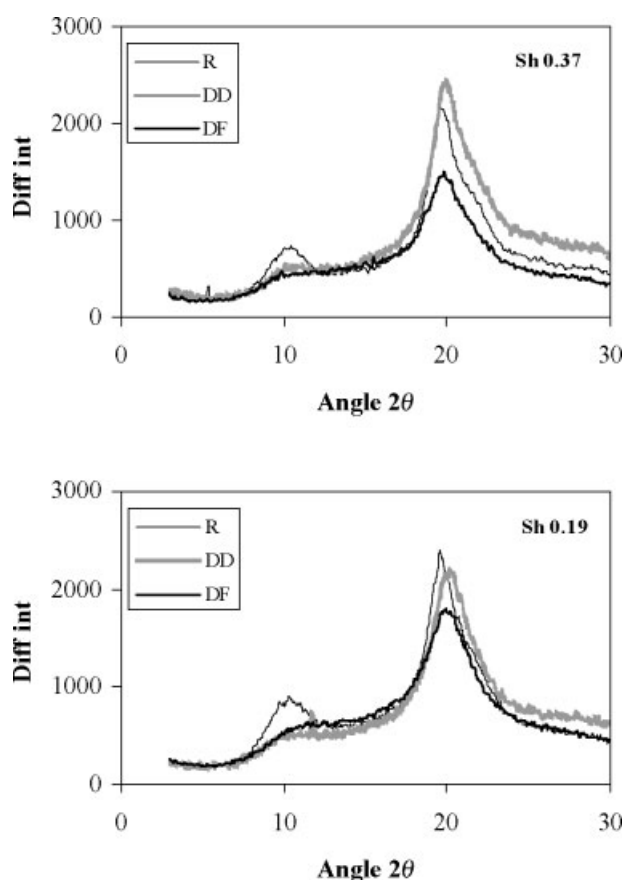


Figure 3. Influence of chitosan conditioning (R, raw material; DD, dissolved and dried at 60 °C; DF, dissolved and freeze-dried) on the X-ray diffraction patterns at different F_A of chitosan from shrimp.

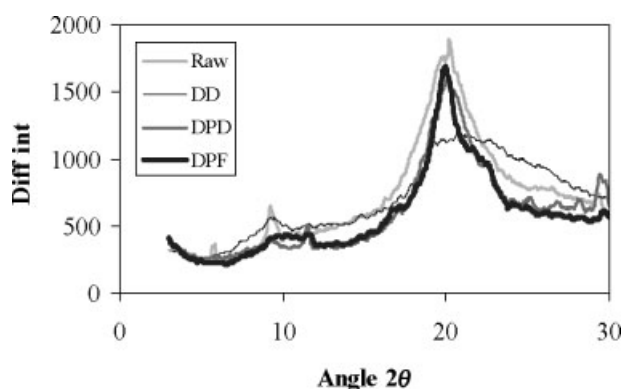


Figure 4. Influence of the intermediary precipitation step on the effect of drying process on the X-ray diffraction pattern of shrimp chitosan (F_A : 0.40).

Figure 4 compares the influence of several treatments on a shrimp chitosan sample (F_A : 0.40): (a) raw material, (b) DD: dissolving and oven-drying, (c) DPD: dissolving, precipitation and oven-drying, (d) DPF: dissolving, precipitation and freeze-drying. The XRD patterns changed considerably according to the treatment used for chitosan conditioning. Surprisingly in this case, the dissolving and oven drying of the solution resulted in a significantly less resolved pattern. After reprecipitation (for both oven-dried and freeze-dried materials) a peak appeared at $2\theta=13^\circ$, and the FWHM decreased progressively in the order: raw > DPD > DPF, however with the DPF sample the shield at $2\theta=23^\circ$ is more marked than for the other samples. Previous experimentation on molybdate sorption using chitosan showed that the presence of a marked peak at $2\theta=23^\circ$ was not favourable to metal sorption.³²

The two crystallinity indices ($Cr I_{area}$ and $Cr I_{peak}$) exhibited inconsistent trends. In the case of $Cr I_{area}$ maximum crystallinity was reached with the oven-dried precipitated material while the freeze-dried precipitated polymer was slightly less crystalline than the raw biopolymer. In the case of $Cr I_{peak}$, the crystallinity was comparable for dried precipitated materials and significantly higher than that of the raw material. Surprisingly, the least crystalline material was the dissolved/oven-dried material. This result is not consistent with the data obtained with other F_A . Whatever the procedure for the calculation of the crystal size, the dissolved/oven-dried material was characterized by the smallest crystal size, while the largest crystal size was obtained with the freeze-dried precipitated polymer.

CONCLUSION

The influence of chitosan chain packing and crystallinity is an important parameter in the characterization of the ability of chitosan to sorb metal ions. These parameters, together with the fraction of acetylation, control the number of available free amine groups and the accessibility of water (for hydration of dry sorbent)

and metal ions to the sorption sites. Consequently, it may affect the sorption equilibrium and the uptake kinetics.

The differences between the XRD patterns of squid and shrimp chitosan samples are not very marked: the main difference is observed in the intensity of the XRD patterns. The deacetylation procedure decreases the differences in the XRD patterns between shrimp and squid chitin samples. Finally, the dissolving/drying procedures hardly influenced the XRD patterns, except in the case of intermediary precipitation steps operated before the drying step.

REFERENCES

- 1 Roberts GAF and Wood FA, Inter-source reproducibility of the chitin deacetylation process, in *Advances in Chitin Sciences*, Vol IV, ed by Peter MG, Domard A and Muzzarelli RAA, Universität Potsdam, Potsdam, Germany, pp 34–39 (2000).
- 2 White SA, Farina PR and Fulton I, *Appl Environ Microb* 38:323–328 (1979).
- 3 Jaworska MM and Szewczyk KW, Chitosan from *Absidia sp.*, in *Advances in Chitin Science*, Vol II, ed by Domard A, Roberts GAF and Vårum KM, Jacques André Publishers, Lyon, France, pp 48–55 (1998).
- 4 Suntornsuk W, Pochanavanich P and Suntornsuk L, *Process Biochem* 37:727–729 (2002).
- 5 Tolaimate A, Desbrières J, Rhazi M, Alagui A, Vincendon M and Vottero P, *Polymer* 41:2463–2469 (2000).
- 6 Zhang M, Haga A, Sekiguchi H and Hirano S, *Int J Biol Macromol* 27:99–105 (2000).
- 7 Roberts GAF, *Chitin Chemistry*, MacMillan, London, UK (1992).
- 8 Kurita K, Hirakawa M, Mori T and Nishiyama Y, Facile preparation of tritylated and trimethylsilylated derivatives starting from β -chitin, in *Advances in Chitin Science*, Vol II, ed by Domard A, Roberts GAF and Vårum KM, Jacques André Publishers, Lyon (France), pp 355–359 (1998).
- 9 Harish Prashanth KV, Kittur FS and Tharanathan RN, *Carbohydr Polym* (in press).
- 10 Ogawa K and Yui T, *Biosci Biotech Biochem* 57:1466–1469 (1993).
- 11 Guibal E, Milot C and Tobin JM, *Ind Eng Chem Res* 37:1454–1463 (1998).
- 12 Jaworska M, Kula K, Chassary P and Guibal E, *Polym Int* (in press).
- 13 Shigemaza Y, Matsura H, Sashiva H and Saimoto H, An improved IR spectroscopic determination of degree of deacetylation of chitin, in *Advances in Chitin Science*, Vol I, ed by Domard A, Jeuniau C, Muzzarelli RAA and Roberts G, Jacques André Publishers, Lyon, France, pp 204–209 (1996).
- 14 Focher B, Beltrame PL, Naggi A and Torri G, *Carbohydr Polym* 12:405–418 (1990).
- 15 Tual C, Espuche E and Domard A, *J Polym Sci B, Polym Phys* 38:1521–1529 (2000).
- 16 Kurita K, Koyama Y and Taniguchi A, *J Appl Polym Sci* 31:1951–1954 (1986).
- 17 Monteiro OAC Jr and Airoidi C, *Int J Biol Macromol* 26:119–128 (1999).
- 18 Ogawa K, *Agric Biol Chem* 55:2375–2379 (1991).
- 19 Piron E, Ph D Thesis, Université Claude Bernard, Lyon I, No 025-97, 220 pp (1997).
- 20 Kim SS, Kim SH and Lee YM, *J Polym Sci B, Polym Phys* 34:2367–2374 (1996).
- 21 Li J, Revol JF and Marchessault RH, *J Appl Polym Sci* 65:373–380 (1997).
- 22 Saito Y, Putaux J-L, Okano T, Gail F and Chanzy H, *Macromolecules* 30:3867–3873 (1997).

- 23 Saito Y, Okano T, Gaill F, Chanzy F and Putaux JL, *Int J Biol Macromol* **28**:81–88 (2000).
- 24 Okuyama K, Noguchi K, Miyazawa T, Yui T and Ogawa K, *Macromolecules* **30**:5849–5855 (1997).
- 25 Okuyama K, Noguchi K, Kanenari M, Egawa T, Osawa K and Ogawa K, *Carbohydr Polym* **41**:237–247 (2000).
- 26 Urbanczyk GW and Lipp-Symonowicz B, *Appl Polym Sci* **51**:2191–2194 (1994).
- 27 Salmon S and Hudson SM, *J Polym Sci B, Polym Phys* **33**:1007–1014 (1995).
- 28 Yui T, Imada K, Okuyama K, Obata Y, Suzuki K and Ogawa K, *Macromolecules* **27**:7601–7605 (1994).
- 29 Sakurai K, Takagi M and Takahashi T, *Sen-I Gakkaishi* **40**:T 246–253 (1984).
- 30 Sakurai K, Takagi M and Takahashi T, *Sen-I Gakkaishi* **41**:T 361–368 (1985).
- 31 Sakurai K, Shibano T and Takahashi T, *Memoires of the Faculty of Engineering, Fukui University* **33**(1):71–91 (1985).
- 32 Milot C, McBrien J, Allen S and Guibal E, *J Appl Polym Sci* **68**:571–580 (1998).