Structure and physicochemical properties of nitrogenated derivatives of cellulose

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Abstract: Nitrogenated derivatives of cellulose, and namely chitin and chitosan, are of commercial interest because of their antibacterial activity, biocompatibility, biodegradability and sorption ability. Structure and some physicochemical properties of these derivatives have been studied by methods of wide-angle X-ray scattering (WAXS), microcalorimetry and sorption. To determine the crystallinity degree of the samples, an improved WAXS method was used based on the calculation of the relationship between integrated intensities of X-ray diffraction from crystalline and amorphous domains. The calculations revealed that the actual degree of crystallinity of initial chitin was 0.72, and of initial chitosan 0.57. After ball-grinding for a short time, the decrease in the crystallinity degree was observed, whereas the prolonged grinding resulted in complete amorphization of the samples. The wetting enthalpy and sorption ability of the samples were inversely proportional to degree of crystallinity. Reduction of the crystallinity degree of the samples promoted increasing of the wetting enthalpy and water sorption. This evidences that mechanism of interaction between the nitrogenated polysaccharides and water is absorption of the water molecules into amorphous domains of the hydrophilic polymers. The crystallinity degree values calculated from results of water vapor sorption and wetting enthalpy were close to the crystallinity degree of the samples obtained by the WAXS method.

Keywords: Chitin, Chitosan, Crystallinity Degree, Wetting Enthalpy, Sorption Ability

1. Introduction

Nitrogenated polysaccharide – chitin, is the most abundant cellulose derivative consisting of 1,4-β-N-acetyl-2-aminodeoxyglucose units [1, 2]. In the nature, chitin is found in exoskeletons of arthropods and insects, shells of mollusks, pens and beaks of cephalopods, cell walls of some fungi, etc. Resources of this polymer are estimated in 100 billion tons [3]. Commercially, chitin can be isolated from shells of crabs, shrimps and lobsters by acidic removal of calcium carbonate followed by alkaline extraction of proteins and bleaching [2]. Chitin is a feedstock for production of another abundant nitrogenated cellulose derivative – chitosan, by means of alkaline deacetylation process. Natural chitosan occurs in cell walls of some fungi, e.g. Mucoraceae [3].

Chitin and in particular chitosan are of commercial interest because of unique properties such as low toxicity, antibacterial activity, biocompatibility, biodegradability and sorption ability [1, 2, 4-7]. Currently these nitrogenated cellulose derivatives have wide range of applications including medicine, cosmetics, biotechnology, biocomposites, papermaking technology, agriculture, etc. Therefore, chitin and chitosan require a detailed structural characterization. The various methods are used for determination of molecular weight, functional acetyl- and amino groups, humidity and content of residual proteins and minerals [2, 6].

Supramolecular structure of these nitrogenated polysaccharides has been studied to a lesser extent. It was found that chitin and chitosan are linear semicrystalline polymers [8, 9]. The linear macromolecules joined by hydrogen bonds form a supermolecular structure of these polymers that consists of nanofibrillar bundles called microfibrils. Each microfibril is built of ordered crystallites and low-ordered non-crystalline (amorphous) domains statistically alternated along the fibril [9]. X-ray studies showed that crystallites of chitin can be in α- and β-polymorph forms [10-12]. The α-form is the most abundant; this form is present in chitin samples isolated...
from crabs, shrimps, krill, lobsters, insects, as well as fungal and yeast cell walls. The rare β-polymorph is found in chitin samples isolated from squid pens and tubeworms and some others sources.

Crystalline unit cell of α-chitin is orthorhombic with space group \(P_2_1_2_1_2\), while β-chitin has monoclinic crystalline unit cell with group \(P_2_1\) [10, 11]. The β-form of chitin is unstable and can undergo an intra-crystalline swelling [13]. Moreover, after some treatments the β-form of chitin is transformed irreversibly into more stable α-polymer [14]. X-ray diffractograms of the chitin samples show two sharp diffraction peaks at \(2\theta\) of about 9-9.5° from (020) planes and 19-19.5° from (110) planes of crystalline unit cells and some weak peaks. Crystalline unit cell of chitosan is also orthorhombic with space group \(P_2_1_2_1_2\); and the X-ray diffractogram of this polymer has two diffraction peaks at \(2\theta\) of about 10-10.5° from (020) planes and 20-20.5° from (110) planes [15]. Thus, as a result of deacetylation of chitin the crystalline peaks are moved to a higher \(2\theta\) angle [16].

However, such structural characteristic as the content of the crystalline phase or the degree of crystallinity of chitin and chitosan has been studied insufficiently. It makes difficult the understanding of real structural organization of these polymers and its change after various treatments. Main reason is that most studies are limited by evaluation of so called index of crystallinity (CrI), based on calculations of the ratio of peaks heights: \(CrI = (I_c - I_a)/I_o\), where \(I_o\) is the maximum height of the crystalline peak and \(I_a\) is height of amorphous scattering [16, 17]. Index of crystallinity shows the comparative content of crystalline fraction in several samples. It may indicate which of the samples has greater crystallinity and which less crystallinity, but it does not disclose the actual degree of crystallinity, i.e., weight part of the crystalline fraction in the polymer. Index of crystallinity of the chitin and chitosan samples was calculated by different ways using heights of crystalline diffraction peaks from (110) or (020) planes and heights of amorphous scattering at \(2\theta\) of 12°, 12.6° or 16° with or without subtraction of the diffuse background scattering [16-21]. The other indexes of crystallinity were proposed based on the dividing the total area of a diffractogram by the crystalline and background areas [22]. Study of crystallinity index of chitin samples showed that depending on the calculation method for the same sample different values of CrI from 0.57 to 0.93 can be obtained [23, 24]. Crystallinity index of the same chitosan sample calculated by different methods can also vary within a wide range from 0.4 to 0.8 [20, 25-27]. Thus, results about the crystallinity of chitin and chitosan samples based on estimation of CrI are doubtful.

To determine the actual degree of crystallinity, the quantitative X-ray phase analysis should be performed, which requires compliance with certain conditions, and namely [28]:

- The scattering areas related to crystalline and non-crystalline domains should be separated from the corrected diffractogram.
- The integrated intensity (area) of crystalline and non-crystalline scatterings should be used to calculate the degree of crystallinity.

Indirect method for estimation of crystallinity can be measurement of hydrophilicity of the polymer samples. Since sorption of water vapor occurs in non-crystalline (amorphous) domains of hydrophilic polymers, more crystalline sample will sorb less water. Investigations of water vapor sorption by chitosan samples revealed that the sorption ability decreases with increasing of deacetylation degree (DD) of the samples [29]. This behavior was explained by the influence of crystallinity of the samples on the sorption ability. Besides, the correlation between crystallinity of the nitrogenated derivatives of cellulose and chitosan samples in order to establish a quantitative relationship between crystallinity degree of the samples and their physicochemical properties.

2. Experimental

2.1. Materials

Purified powders of chitin isolated from crab shells and chitosan (MW=400 kDa; DD=85%) were acquired from Sigma-Aldrich. To reduce the crystallinity, the initial samples were ground in laboratory ball-mill using ceramic balls at 250 rpm for 5 and 24 hours.

2.2. Wide-Angle X-Ray Scattering (WAXS)

The dry powders of the equal mass (250 mg) are pressed into tablets (diameter 15 mm, thickness 1.5 mm) that were used for WAXS-experiments. Diffractograms of the samples were recorded in the \(2\theta\) angle range from 5 to 50° using a Rigaku-Ultima Plus diffractometer (CuK\(_{\alpha}\) - radiation, \(\lambda=0.15418\) nm). After recording of the diffractograms, the parasitic background (bg) caused by air, apparatus, Compton scattering, thermal agitation of atoms and molecules and distortions of crystalline lattice was subtracted. To correct of the diffractogram, the X-ray intensities were divided into the correction coefficient, \(K(\theta)\), that includes the Lorentz-polarization factor (LP) and initial intensity of the X-ray beam (J): \(K(\theta) = JLP\) [28]. Further, the total integrated intensity (total area) of the corrected diffractogram was separated into areas of crystalline (Cr) and amorphous (Am) scattering (Fig. 1, 2). For this purpose, a similar transfer of the profile of X-ray scattering of the amorphous sample was carried out (Fig. 3). Finally, the actual degree of crystallinity \((\chi)\) of the sample was calculated, as follows:

\[
X = \frac{I_c}{d\theta} d\theta / I_o d\theta = F_c/(F_c + F_{am})
\]

where \(I_o\) is total intensity of the corrected diffractogram after subtraction of the parasitic background (bg); \(I_c\) is intensity
of the crystalline scattering; $F_{cr}$ is area of the crystalline scattering; $F_{am}$ is area of the amorphous scattering.

2.3. Determination of Wetting Enthalpy

Wetting enthalpy of cellulose derivatives was studied at 25 °C using microcalorimeter of Calvet-type [31]. The polymer sample (100 mg) was placed into a special glass micro-ampoule, dried at 105 °C up to constant weight, sealed and cooled in a vacuum desiccator. Distilled water (5 ml) was poured into two cells of the microcalorimeter. The hermetically closed ampoule containing the dry sample was put into the one cell, while empty ampoule was placed into another cell of the microcalorimeter. The microcalorimeter was thermostated at 25 °C to achieve an equilibrium state. Then, the ampoule containing the sample was crushed to ensure contact of the sample with water and measuring the exothermal heat effect ($Q$). The wetting enthalpy ($H$) was calculated using the equation (2):

$$H = \frac{Q}{m}$$

where $Q$ – heat of interaction; $m$ – weight of dry sample.

2.4. Water Vapor Sorption

The sorption experiments were carried out at 25°C using a vacuum Mac-Ben apparatus having helical spring quartz scales [32]. Prior to starting the experiments the samples were dried at 105°C up to constant weight and additionally degassed in the sorption device.

3. Results and Discussion

X-ray diffractogram of initial chitin sample was typical for α-polymorph having two expressed peaks at 2θ of 9.2° and 19.0° (Fig. 1, 4). These peaks appear as a result of X-ray diffraction from (020) and (110) planes of crystalline cell with inter-plane distances of 0.96 nm and 0.47 nm, correspondingly. Initial chitosan sample gave the diffractogram having a weak peak at 2θ of 10° and more intense peak at 2θ of 20° that caused by diffraction from (020) and (110) planes of crystalline cell with inter-plane distances of 0.88 nm and 0.45 nm, correspondingly (Fig. 2, 5).

After ball-grinding of the initial samples for a relative short time (for 5 h), the intensity of the diffraction peaks decreased that indicated on partial decrystallization of the samples (Fig. 4, 5). Prolonged grinding (for 24 h) resulted in complete decrystallization of the samples and appearance wide diffractograms having one maximum at 2θ ≈ 20° typical for amorphous phase state of the polymers (Fig. 3).
Calculations carried out by the equation (1) based on the improved X-ray method revealed that actual degree of crystallinity of initial chitin was 0.72, and of initial chitosan sample was 0.57 (Table 1). Short-time grinding (for 5 h) caused a decrease in the crystallinity degree of the samples to 0.40-0.45, whereas prolonged grinding (for 24 h) resulted in the complete amorphization of the samples.

Table 1. Crystallinity degree (X) of the samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitin</td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>0.72</td>
</tr>
<tr>
<td>Ground for 5 h</td>
<td>0.45</td>
</tr>
<tr>
<td>Ground for 24 h</td>
<td>0</td>
</tr>
<tr>
<td>Chitosan</td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>0.57</td>
</tr>
<tr>
<td>Ground for 5 h</td>
<td>0.40</td>
</tr>
<tr>
<td>Ground for 24 h</td>
<td>0</td>
</tr>
</tbody>
</table>

Microcalorimetric studies showed that wetting enthalpy of the chitin and chitosan samples was inversely proportional to crystallinity degree (Table 2).

Table 2. Wetting enthalpy (H) of the samples having different crystallinity degree (X)

<table>
<thead>
<tr>
<th>Sample</th>
<th>X</th>
<th>H, kJ/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>0.72</td>
<td>38</td>
</tr>
<tr>
<td>Ground for 5 h</td>
<td>0.45</td>
<td>75</td>
</tr>
<tr>
<td>Ground for 24 h</td>
<td>0</td>
<td>134</td>
</tr>
<tr>
<td>Chitosan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>0.57</td>
<td>76</td>
</tr>
<tr>
<td>Ground for 5 h</td>
<td>0.40</td>
<td>101</td>
</tr>
<tr>
<td>Ground for 24 h</td>
<td>0</td>
<td>174</td>
</tr>
</tbody>
</table>

Using results of wetting enthalpy, the crystallinity degree of the samples can be calculated, as follows:

\[ X_H = 1 - \left( \frac{H}{H_a} \right) \]  \hspace{1cm} (3)

where \( H_a \) is wetting enthalpy for complete amorphized sample.

Sorption of vapors is usually described through isotherms that express the relative amount of sorbate in the sorbent (\( A, \ g/g \)) as a function of relative vapor pressure (\( \varphi = p/p_o \)) at a constant temperature (e.g. 25°C). The experiments showed that isotherms of water vapor sorption by chitin and chitosan samples had a sigmoidal shape similar to sorption isotherms of this sorbate by cellulose (Fig. 6, 7). The sorption ability of more crystalline initial samples was less than of partially or completely amorphized samples (Fig. 8, 9). Thus increasing of the crystallinity degree leads to reducing in sorption ability of the sample. Comparison of sorption isotherms for amorphous samples showed that the sorption ability of chitin containing hydrophobic acetyl groups was lower than chitosan containing only hydrophilic hydroxyl and amine groups.

In order to linearize the sigmoidal isotherms, the following thermodynamic equation can be applied [32]:

\[ \frac{1}{A} = \frac{1}{A_o} - (K/A_o) \ln \varphi \]  \hspace{1cm} (4)

where \( A_o \) is maximal sorption value at \( \varphi=1 \); \( K \) is coefficient.
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Figure 8. Sorption ability of the chitin samples as function of crystallinity degree at φ=0.5

Figure 9. Sorption ability of the chitosan samples as function of crystallinity degree at φ=0.5

The linearized isotherms of water vapor sorption by amorphized polymer samples are shown, for example, on Fig. 10. From the linearized isotherms the sorption parameters $A_o$ and $K$ were found for the amorphized samples. This permits to calculate the sigmoidal isotherms using the following equation:

$$A = A_o \frac{(1-X_s)}{(1 - K \ln \phi)}$$  \hspace{1cm} (5)

where for chitin samples $A_o=0.4 \text{ g/g}$; $K=2.7$, while for chitosan samples with DD=85% $A_o=0.5 \text{ g/g}$; $K=2.7$; $X_s$ is degree of crystallinity.

Figure 10. Linearized sorption isotherms of amorphized chitin (1) and chitosan (2) samples

By means of eq. (5) degree of crystallinity ($X_s$) of the sample can be determined from sorption experiments. For this purpose it is convenient to use the sorption value $A_{0.5}$ (g/g) at the relative pressure of vapors $\phi = 0.5$. Then:

$$X_s = 1 - n A_{0.5}$$ \hspace{1cm} (6)

where for chitin samples coefficient $n=7.15$ and for chitosan samples with DD=85% coefficient $n=5.65$.

The crystallinity degree calculated from results of wetting enthalpy and water vapor sorption correlates with this structural characteristic obtained by the WAXS method (Table 3).

Table 3. Crystallinity degree of the samples obtained by WAXS ($X$), microcalorimetry ($X_H$) and sorption ($X_s$)

<table>
<thead>
<tr>
<th>Sample</th>
<th>$X$</th>
<th>$X_H$</th>
<th>$X_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitin</td>
<td>0.72</td>
<td>0.71</td>
<td>0.74</td>
</tr>
<tr>
<td>Initial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground for 5 h</td>
<td>0.45</td>
<td>0.44</td>
<td>0.46</td>
</tr>
<tr>
<td>Chitosan</td>
<td>0.57</td>
<td>0.56</td>
<td>0.58</td>
</tr>
<tr>
<td>Initial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ground for 5 h</td>
<td>0.40</td>
<td>0.42</td>
<td>0.38</td>
</tr>
</tbody>
</table>

As follows from the investigations, the wetting enthalpy and sorption ability of amorphized samples is higher than of crystalline samples.

Thus, reduction of the crystallinity degree contributes to increase of the wetting enthalpy and sorption value. This evidences that mechanism of water interaction is absorption of the water molecules into amorphous domains of the hydrophilic polymers.

4. Conclusion

Crystallinity is an important structural characteristic influencing the various properties of polymers. This characteristic permits also to understand the supramolecular structure of the polymers. Unfortunately, the crystallinity of chitin and chitosan samples has been measured by insufficiently correct methods using the ratio of heights of X-ray peaks. For this purpose, the heights of crystalline diffraction peaks from (110) or (020) planes and heights of amorphous scattering at 2θ of 12°, 12.6° or 16° were used. However, the experiments showed that the maximum of amorphous X-ray scattering is observed at 2θ ≈20°, but not in the 2θ range of 12-16°. Besides, evaluation of the crystallinity from the ratio of peaks heights is not justified theoretically because the peak height is not proportional to the content of crystalline or amorphous phase. The law of phase proportionality is observed only, when integral intensities (areas) of the X-diffraction are used [28]. In this study to determine the crystallinity an improved method was used based on the calculation of the relationship between integrated intensities of X-ray diffraction from crystalline and amorphous phases. The calculations revealed that actual degree of crystallinity of initial chitin was 0.72, and of initial chitosan sample was 0.57. After ball-grinding for a short time, the decrease of the crystallinity degree of the samples was observed, whereas the prolonged grinding resulted in
the complete amorphization of the samples. Isotherms sorption of water vapor by chitin and chitosan samples had a sigmoidal shape, which can be described by a thermodynamic equation:

\[ A = A_o \frac{(1-X_o)}{(1-K \ln \phi)} \]

where \( \phi \) is relative vapor pressure; \( A_o \) is maximal sorption value at \( \phi = 1 \); \( X_o \) is degree of crystallinity; \( K \) is coefficient.

As follows from the investigations, the wetting enthalpy and sorption ability of the samples were inversely proportional to their degree of crystallinity. Reduction of the crystallinity degree of the samples promoted to increase of wetting enthalpy and sorption value. This evidences that mechanism of water interaction is absorption of the water molecules into amorphous domains of the hydrophilic polymers. Experiments showed that the sorption ability and wetting enthalpy of chitin containing hydrophobic acetyl groups were lower than chitosan containing only hydrophilic hydroxyl and amine groups. The crystallinity degree calculated from results of wetting enthalpy and water vapor sorption was close to the crystallinity degree of the samples obtained by the WAXS method.

References


