

## 14. Determination of Hydrogel Chitosan Membrane Structure

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### 1. Introduction

Main parameters that determine properties of chitosan, apart from molecular mass and deacetylation degree is polymer crystallinity. The chitosan is a derivative of chitin, which is a linear, high molecular weight, crystalline polysaccharide consisting of  $\beta$ -(1 $\rightarrow$ 4) linked N-acetyl-D-glucosamine. X-ray diffraction analysis revealed that chitin is a polymorphic substance that occurs in three different crystalline modifications, termed  $\alpha$ -,  $\beta$ - and  $\gamma$ -chitin. They mainly differ in the degree of hydration, in the size of the unit cell and in the number of chitin chains per unit cell. The most abundant and easily accessible form is  $\alpha$ -chitin, where molecules are aligned in an antiparallel fashion. This molecular arrangement is favourable for the formation of strong intermolecular hydrogen bonding. The  $\alpha$ -chitin is the most stable form. In the  $\beta$ -chitin, molecules are packed in a parallel arrangement, leading to weaker intermolecular forces. In the  $\gamma$ -chitin form sets of two parallel strands alternate with single antiparallel strands [1-2].

The chitosan is also crystalline and shows polymorphism depending on its physical state. Recently, true crystal structure and configuration of chitosan is intensively investigated. The structures for different forms including an anhydrous form, a hydrated form and various salts were obtained by X-ray diffraction analyses [3-7]. The small angle X-ray diffraction studies carried out on chitosan powder, obtained from crab shells, show regular packing of the molecules in parallel bundles. The data indicate

that the interactions of chitosan macromolecules along an *b*-axis give rise to a fibrous structure [3]. Research performed by Ogawa and Yui [4 - 5] was dedicated to three chitosan forms (hydrated, anhydrous crystal and non-crystal). The hydrated form of chitosan reveals a sharp peak at the angle  $2\theta = 10.4^\circ$  and a weak peak at  $2\theta = 20 - 22^\circ$ . The anhydrous form of chitosan is characterised by a main sharp peak at  $2\theta = 15^\circ$  and an additional one at the angle  $2\theta = 20^\circ$ . An amorphous form has only background raised by about  $20^\circ$  at  $2\theta$ . In turn, studies of Twu [6] refer to crystallinity of the chitosan formed as scaffolds. The scaffolds were formed by electrolysis from chitosan of different molecular mass (1050, 965, 615 kDa) and using different solvents (acetic and formic acid). Crystallinity of the chitosan scaffolds is lower than crystallinity of initial polymers and it drops with an increase of the acid concentration. In diffractograms distinct peaks were obtained at  $2\theta = 20^\circ$ . With an increase of the acid concentration the peaks lowered and shifted towards bigger angles. Trung [7] studied the crystallinity of chitosan of constant molecular mass and different deacetylation degrees. The X-ray diffractograms of all chitosan samples show two peaks approximately at  $2\theta = 10^\circ$  and  $2\theta = 20^\circ$ . For chitosan of lower deacetylation degree their intensity is lower and the peaks are weak, which shows that chitosan of a higher deacetylation degree is characterised by higher crystallinity. On the other hand, membranes produced from solutions of these chitosans (0.5% solutions after drying were coagulated in NaOH) have lower crystallinity than initial polymers. But, like in the case of the initial polymer, the crystallinity of membranes decreases with a reduction of the deacetylation degree.

Our study shows that both the parameters which characterise the polymer (deacetylation degree) and these related to the physical structure (powder, membrane, scaffolds) have a significant effect on crystallinity. Research of the crystallinity of hydrogel membranes formed from high viscous solutions of chitosan acetate and lactate is discussed in the paper. Membranes were made from chitosan of molecular mass 218 kDa (MIR) and 500 kDa (Fluka).

## 2. Experimental

### 2.1. Materials

Chitosan of molecular mass 218 kD and deacetylation degree 90% produced by MIR and with molecular weight 500 kD and deacetylation degree 74% produced by Fluka.

### 2.2. Membrane formation

Experiments were made with hydrogel chitosan membranes. The membrane-forming solutions were chitosan salts in the form of acetate and lactate with different polymer concentrations. Chitosan membranes with 90% deacetylation degree (MIR) were produced from chitosan acetate and lactate containing from 6 to 12% polymer. Chitosan membranes with 74% deacetylation degree (Fluka) were managed to be formed from solutions that contained 7% polymer (5-7%) at the most. The amount of solvent was chosen stoichiometrically to the content of amino groups in a chitosan molecule. Co-

agulation was carried out up to 5% NaOH and 10% Na<sub>2</sub>CO<sub>3</sub>. After coagulation the membranes were washed with demineralized water to pH=7.

To maintain a porous structure, the membranes were plastified in a water solution of glycerin (1:1).

### **2.3. Structural characteristics**

The structural characteristics were based on the analysis of XRD spectra. The X-ray diffraction patterns were determined using a wide-angle X-ray Simens D5000 diffractometer and the K<sub>α,Cu</sub> radiation.

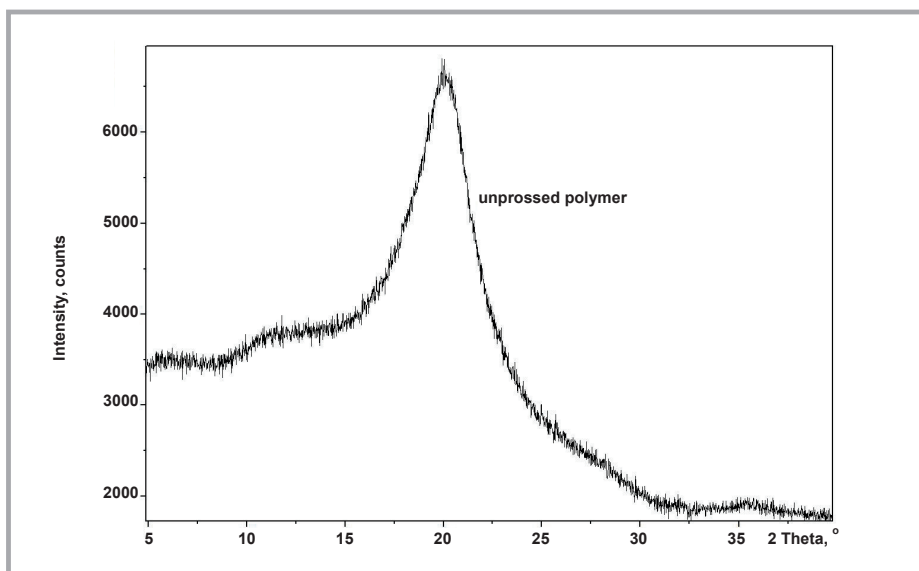
## **3. Results**

Studies were divided into two parts. The first one covered membranes formed from Fluka chitosan, while the other one from MIR chitosan.

A diffractogram of the Fluka chitosan is shown in Figure 1, and diffractograms of the membranes formed from Fluka chitosan in Figures 2–4. By analogy, for MIR chitosan the diffractogram, and diffractograms of the membranes produced from MIR chitosan are presented in Figure 5.

### **3.1 Fluka Chitosan**

In the polymer diffractogram only one peak is clearly visible, whose maximum occurs at the angle  $2\theta = 20^\circ$ . Slight peaks are also visible at the angles  $2\theta = 29^\circ$  and  $2\theta = 11^\circ$ .

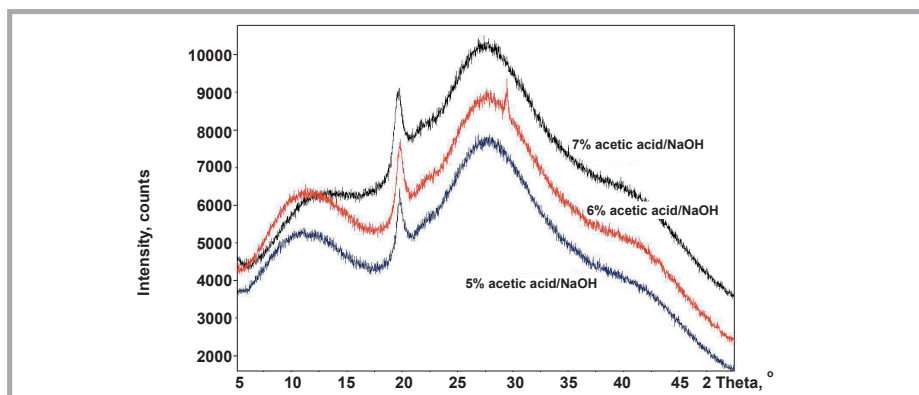


*Figure 1. Diffractogram of Fluka chitosan.*

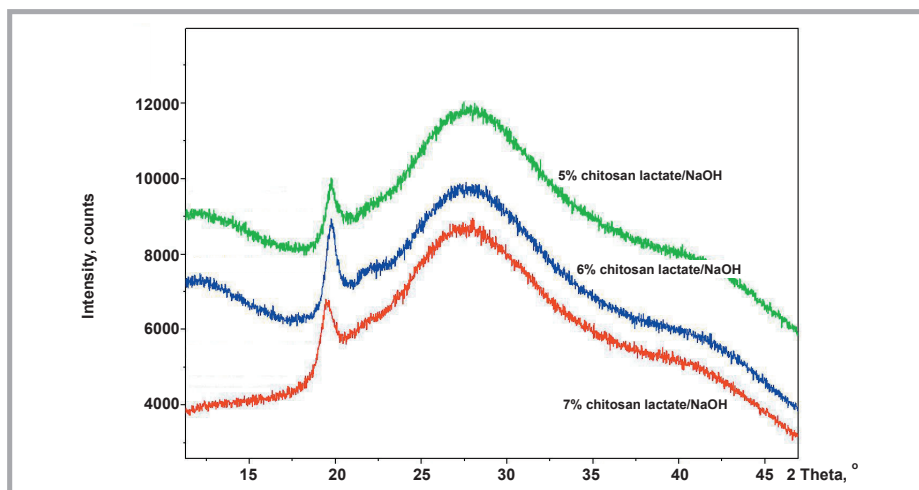
It follows from the above that initial chitosan as a polymer with low crystallinity index is a rather amorphous body.

Upon transformation of the chitosan into the form of hydrogel membranes, the crystalline structure changes.

The effect of polymer concentration on structural properties of the membranes produced from acetate and lactate is shown in Figure 2a and b, the effect of a coagulant in Figure 3a and b and the influence of plasticization with glycerin in Figure 4.



**Figure 2a.** X-ray diffractograms depending on polymer concentration in the solution chitosan acetate.



**Figure 2 b.** X-ray diffractograms depending on polymer concentration in the solution chitosan lactate.

Diffraction patterns of hydrogel membranes differ from the diffraction pattern of initial chitosan, hence ordering of glucosamine molecules in the hydrogel is different than in the initial polymer. In the diffraction patterns of hydrogel membranes formed from chitosan acetate three distinct peaks with maxima occurring at the angles  $2\theta = 12, 20$  and  $28^\circ$ , and a slight peak at  $2\theta = 42^\circ$  appear.

Diffraction patterns obtained for membranes with polymer concentration equal to 5-6%, do not differ significantly from one another. Three main maxima are observed in the range of the same angles. Their intensity is also similar. Hence, no change in the structure ordering in dependence of the polymer concentration is observed. For membranes formed from 7% solution of chitosan acetate a decrease of the peak intensity is reported at the angle  $2\theta = 12^\circ$ .

Diffraction patterns of the membranes formed from lactate are similar to the diffraction patterns of membranes produced from chitosan acetate. Three distinct peaks appear with maxima at the angles  $2\theta = 12, 20$  and  $28^\circ$ .

Diffraction patterns obtained for membranes with polymer concentrations ranging from 5 to 6% overlap. Three main maxima are visible in the range of the same angles. Their intensity is also similar. A slightly lower is the peak for membranes formed from 5% solution of lactate at the angle  $2\theta = 20$ . Therefore, no change of structure ordering in dependence of polymer concentration is observed. A difference is visible for membranes formed from 7% solution. One can see peaks at  $2\theta = 20$  and  $28^\circ$ , a peak at  $2\theta = 12$  disappears, and at the angle  $2\theta = 20^\circ$  it is shifted towards smaller angles.

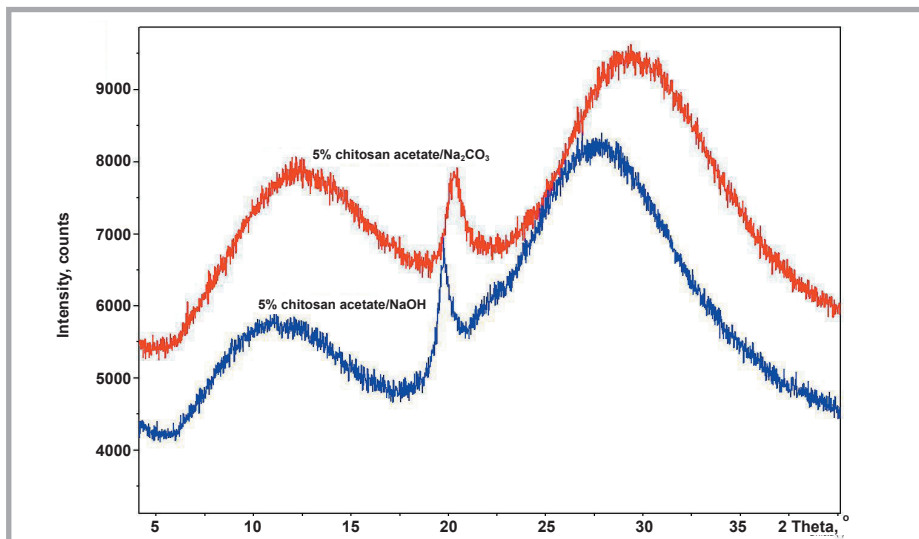
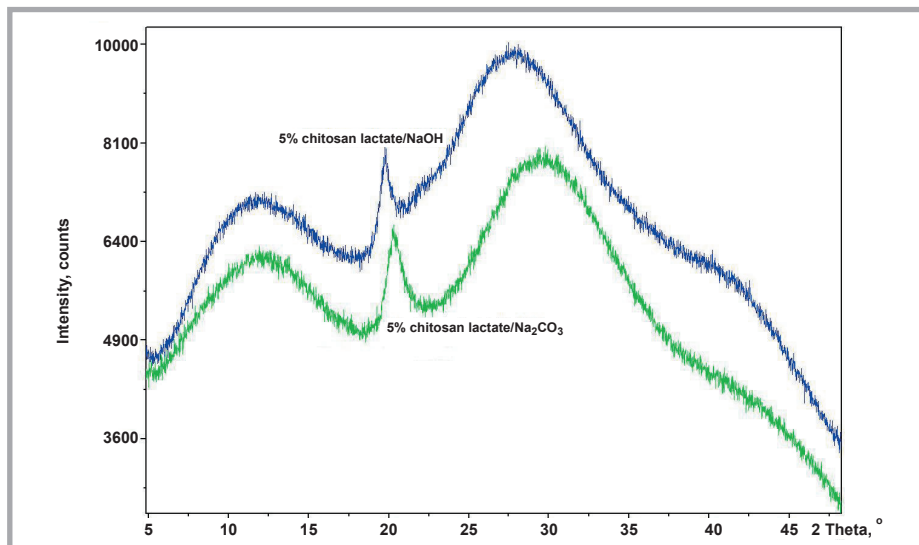
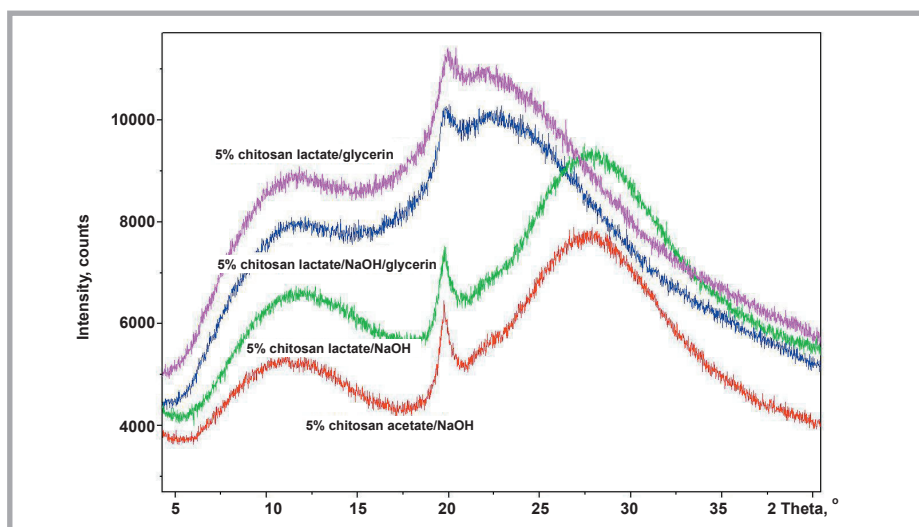


Figure 3 a. X-ray diffraction patterns depending on coagulation types chitosan acetate.



**Figure 3 b.** X-ray diffractograms depending on coagulation types chitosan lactate.

Coagulation in sodium hydroxide NaOH and sodium carbonate Na<sub>2</sub>CO<sub>3</sub> only slightly affect the ordering. Peaks are observed at the same values of 2 $\theta$ . In the case of coagulation in NaOH, there is a slight shift of all peaks towards smaller angles (by 2 - 3°). This can be related to higher ordering of the amorphous phase resulting in greater strength of the membranes coagulated in NaOH .



**Figure 4.** The effect of plasticization with glycerin.

When glycerin is used as a plastifier, significant differences are observed in diffractograms. A broad peak at the angle  $2\theta = 28^\circ$  shifts towards lower angles and approaches a maximum occurring at the angle  $2\theta = 20^\circ$ . The intensity of peaks is much higher.

### 3.2. MIR Chitosan

A quite different character have the membranes formed from MIR chitosan with the use of chitosan acetate as a film-forming solution.

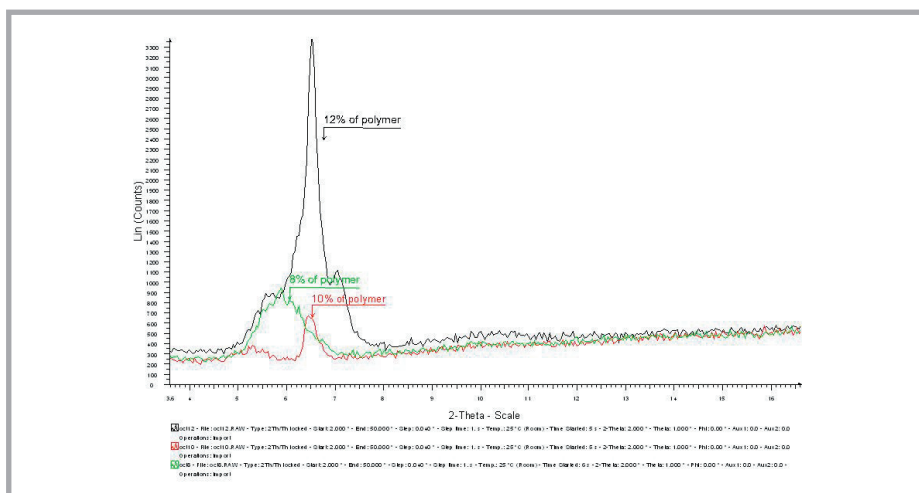


Figure 5 a. X-ray diffractograms depend on polymer concentration in the solution  $2\theta = 0 - 10^\circ$ .

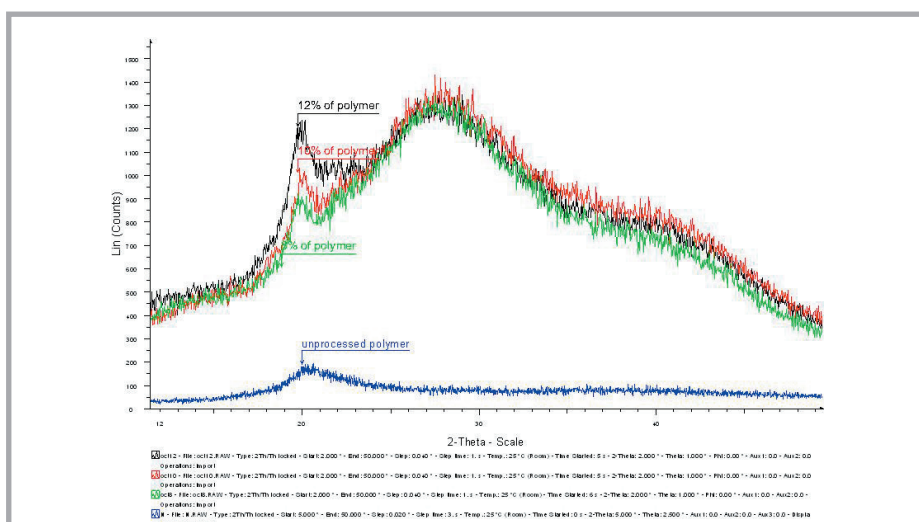


Figure 5 b. X-ray diffractograms depend on polymer concentration in the solution  $2\theta = 10 - 28^\circ$ .

The XRD patterns showed that crystallinity of the membranes depends on polymer concentration in the solution. Hydrogel chitosan membranes reveal better ordering than the initial polymer. Pure chitosan used in this study exhibits rather amorphous properties. Membranes formed from a polymer of concentration below 6% are amorphous, like pure chitosan. This is revealed by a broad peak with a maximum at the angle  $2\theta = 28^\circ$  (in comparison to the diffractogram of pure chitosan, shifting towards higher angles is observed). For the membranes formed from solutions of concentrations exceeding 6%, the XRD patterns are characterized by broad peaks around  $2\theta = 5 - 8^\circ$  and  $2\theta = 19 - 20^\circ$ . Membranes formed from 8% solution have untypical properties, namely they are characterized by higher peak intensity in the low angle range than the membranes formed from 10% polymer solution. Moreover, this peak is shifted towards lower angles.

The above study seems to confirm that membranes with more crystalline structure can be obtained from 8% solutions.

#### 4. Concluding remarks

- Transformation of chitosan into hydrogel form changes its crystalline structure. This is observed both for Fluka and MIR chitosan.
- Ordering of the membrane structure depends mainly on polymer type: molecular weight and deacetylation degree.
- The structure depends also on the polymer concentration in the film-forming solution.
  - For membranes formed from a polymer with low deacetylation degree (Fluka) from solutions at concentrations up to 6%, no changes in the structure induced by a change of solvent are observed. Membranes reveal ordering only in the amorphous phase.
  - Membranes formed from chitosan of high deacetylation degree (MIR) from solutions containing 6% polymer also reveal ordering only in the amorphous phase.
  - At the concentration above 6%, differences in the structure ordering are obtained:
    - For the membranes formed from chitosan with a lower deacetylation degree (70%) (Fluka), the peak decays at the angle  $2\theta = 12$ , the peak at the angle  $2\theta = 20$  is shifted toward lower values.
    - For the membranes formed from chitosan with a higher deacetylation degree (MIR), peaks appear within the range of small angles, which may suggest ordering of the structure on molecular level. From chitosan with high deacetylation degree and proper polymer concentration hydrogel membranes with high ordering can be produced.
- The structure is changed due to plasticization in glycerin.

#### 5. References

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