The Influence of Processing Terms of Chitosan Membranes Made of Differently Deacetylated Chitin on the Crystalline Structure of Membranes

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SYNOPSIS

The crystalline structure of chitosan membranes made of a high degree of (90%) and low degree of (60%) deacetylated chitin by various regeneration processes was studied. The results of X-ray and IR spectroscopy investigations testify that the type of crystalline structure, the degree of crystallinity, and the average lateral crystallite size depend basically on the deacetylation degree of the chitin utilized in preparing the membranes. © 1994 John Wiley & Sons, Inc.

INTRODUCTION

Chitosan reveals very distinctly pronounced bioactivity and hemocompatibility.[1-6] Therefore, among other applications, it is used in the coating of artificial blood vessels made of polyester fibers. The coating of the artificial blood vessel surface with a chitosan membrane seals up the vessel and, combined with the antitrombosic properties and the hemocompatibility of chitosan, facilitates surgical treatment and the successive adaptation of the artificial vessel to the human organism. The results of biomedical investigations show that chitosan membranes coated on artificial blood vessels exhibit differentiated bioactivity.

The possibility of utilizing differently deacetylated chitin, which results, in turn, in chitosan with different molecular weights, and the possibility to apply various membrane manufacturing techniques give rise to the presumption that the differences in the bioactivity of membranes may be connected with their molecular and supermolecular structure. Within the range of the supermolecular structure, it can be supposed that the crystalline structure of the membrane should play an important role. Based on this assumption, the crystalline structure of membranes manufactured in various ways, made of chitosan with a high degree of chitin deacetylation (90%) and a low degree of deacetylation (60%), has been investigated.

Both kinds of chitosan used have been produced by the acetylation of krill chitin (Euphausia Superba), applying concentrated NaOH solution. The values of the deacetylation degree have been established by titration of the product of distillation of the chitosan specimen in 85% (m/m) orthophosphoric acid performed at 160°C. The desired degree of deacetylation was calculated from the formula

$$\text{Deacetylation degree} = 100 - 2.03 \cdot V/m(\%)$$

where V is the volume of 0.1 mol/L NaOH used during titration (mL); m, the mass of the chitosan specimen (g); and 2.03, the correlation coefficient resulting from taking into account the molecular weight of chitin. The ascertained values of the degree of deacetylation are the averages of three independent measurements.

Membranes of 30–50 μm thickness have been prepared by casting from 1% chitosan solutions in formic and acetic acids. After solidifying, the membranes underwent a successive regeneration treatment in two kinds of regenerating baths—a mixture of NH_4OH and C_2H_5OH and a mixture of NaOH and C_2H_5OH. The considered variants of the membranes are outlined in Table I.

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Table I Considered Chitosan Membranes

Sample Symbol	Degree of Deacetylation (%)	Applicated Solvent	Applicated Regenerating Bath
1	60	HCOOH	$NH_4OH + C_2H_5OH$
2	60	HCOOH	$NaOH + C_2H_5OH$
3	60	CH_3COOH	$NH_4OH + C_2H_5OH$
4	60	CH_3COOH	$NaOH + C_2H_5OH$
5	90	HCOOH	$NH_4OH + C_2H_5OH$
6	90	HCOOH	$NaOH + C_2H_5OH$
7	90	CH_3COOH	$NH_4OH + C_2H_5OH$
8	90	CH_3COOH	$NaOH + C_2H_5OH$

The crystalline structure was examined using wide-angle X-ray diffraction and IR spectroscopy. These investigations involved the identification of the crystallographic type of lattice, determination of crystallinity degree, evaluation of the average lateral crystallite size, and the assessment of the presence of spherilitic crystalline aggregations.

RESULTS AND DISCUSSION

Identification of the Crystallographic Type of the Crystalline Structure

Conclusions about the crystallographic type of the crystalline structure were based on the intensity distribution $I = f(2Q)$ of the diffracted and scattered X-ray radiation. The $I = f(2Q)$ curves achieved for particular kinds of membranes show marked differences. The occurrence of two qualitatively different types of $I = f(2Q)$ curves could be seen (Figs. 1 and 2). The first one pertains to membranes made of chitosan with a low deacetylation degree of chitin (60%), and the second one refers to membranes made of higher deacetylated chitin (90%).

The analysis of the angular positions of the diffraction peaks give evidence that in the case of membranes with 60% deacetylation the crystal structure of type II, according to the designation introduced by Samuels,[7] occurs. In the case of membranes with 90% deacetylation, the crystal structure refers to a mixture of types I and II (Ref. 7) of the crystalline chitosan.

The findings from X-ray examination were fully corroborated by the results of IR spectroscopy investigations (Table II). It was ascertained that spectrograms of membranes with 60% deacetylation displayed the 1670 cm^{-1} absorption band characteristic of type I crystal structure,[7] but did not display the bands at 1350 and 760 cm^{-1} relevant for type II crystal structure.[7] On the contrary, the IR spectrograms of membranes with 90% deacetylation comprised all the absorption bands mentioned above.

The X-ray intensity distribution curves obtained exhibit only a small number of diffraction peaks and do not include reflections of higher order, which leads us to conclude that in the case of all variants of the membranes investigated the perfection of lattice arrangement is very poor. The investigations carried out also prove that the technology of membrane manufacture (kind of solvent and kind of regenerating bath) does not influence the type of the crystal structure established.

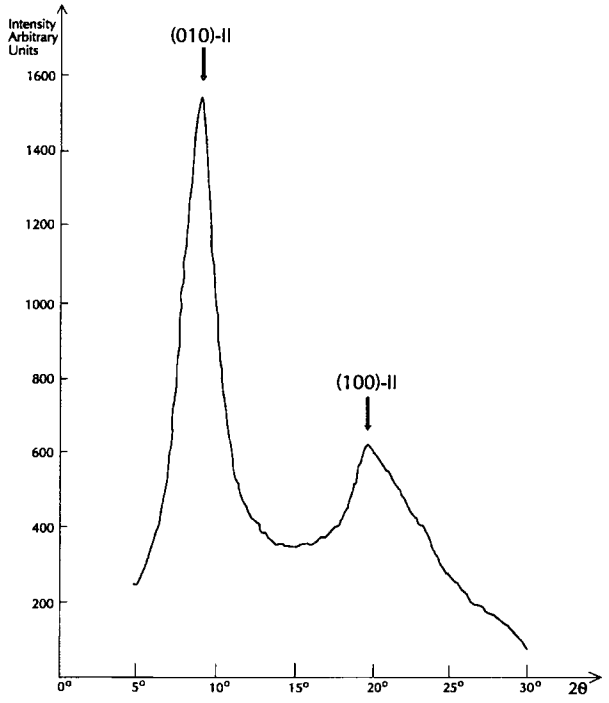

Figure 1 X-ray intensity distribution $I = f(2Q)$ of chitosan membranes with 60% deacetylation; type I crystal structure.[7]

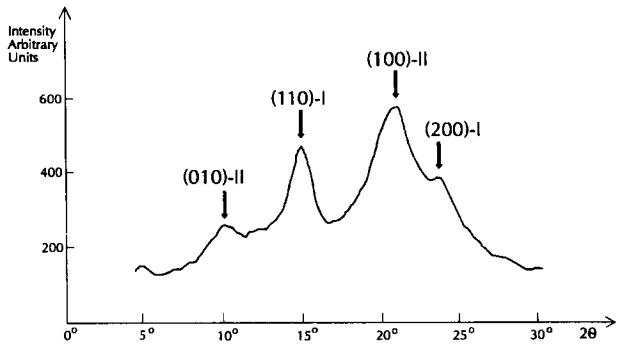

Figure 2 X-ray intensity distribution $I = f(2Q)$ of chitosan membranes with 90% deacetylation; types I and II crystal structure.[7]

The complementary polarizing-interferometric examination performed revealed a lack of Maltese cross patterns in the observed images of membranes, which proved the absence of spherilites in them. This allows us to suppose that during solidification of the membranes spherilitical crystallization of the chitosan does not proceed.

Quantitative Study of the Crystal Structure

The chitosan membranes considered exhibit essential differentiation with respect to crystallizing ability. The values of crystallinity degree and of average lateral crystallite size achieved from X-ray diffraction measurements are collected in Table III. The degree of crystallinity has been evaluated on the basis of the integral diffracted intensities of chitosan samples and standard reference sample (cellulose I) by regarding the differences in X-ray scattering ability of both kinds of specimens according to the procedure of Hermans and Weidinger.[8] The average lateral crystallite sizes have been calculated on the basis of the measured half-width of the diffraction peaks 010, 110, and 100 of the WAXS diagrams from the fundamental Scherrer expression.

The results testify that for membranes with a 60% deacetylation the crystallinity degree is noticeably higher (0.35–0.41) than for membranes with 90% deacetylation (0.15–0.18). Crystallization efficiency is also, but only to a small extent, influenced by the manufacturing technique used for the membranes. Preparation from HCOOH solutions leads to a higher degree of crystallinity than does preparation from CH_3COOH solutions. Such dependence is evidently pronounced in the case of the successive use of a $NH_4OH + C_2H_5OH$ mixture used as the regenerating bath. In the case of the $NaOH + C_2H_5OH$ mixture used as the regenerating bath, the influence of the kind of solvent used does not play a visible role.

The degree of deacetylation determines not only the degree of crystallinity, but also influences the average lateral crystallite size, i.e., the "crystalline granularity" of the membranes. In the light of data collected in Table III, it is evident that the average lateral crystallite dimensions are larger in the case of membranes with 60% deacetylation than for membranes with 90% deacetylation. Additionally, the average crystallite size depends on the kind of the solvent used. For membranes with 60% deacetylation, the average size is larger in the case of applying HCOOH as the solvent than in the case of CH_3COOH. For membranes with 90% deacetylation, the relationship is the opposite.

Appraisal of the Molecular Structure

Within the limits of a comprehensive IR spectroscopy study, particular attention was devoted to the presence of accessible OH groups, which determine the chemical reactivity and, also, as it can be assumed, the bioactivity of the chitosan membranes.

Table II Results of IR Spectroscopy Examination

	Extinction Values of the Absorption Band E						
Sample Symbol	1670 cm^{-1}	1580 cm^{-1}	E1670/ E1580	1350 cm^{-1}	760 cm^{-1}	1380 cm^{-1}	1320 cm^{-1}
1	0.591	0.238	2.48	Absent	Absent	0.237	0.202
2	0.627	0.280	2.23	Absent	Absent	0.283	0.215
3	0.618	0.327	1.89	Absent	Absent	0.253	0.242
4	0.628	0.292	2.15	Absent	Absent	0.234	0.194
5	0.213	0.222	0.95	0.202	Vestigial	0.094	0.097
6	0.236	0.374	0.63	0.219	0.024	0.164	0.153
7	0.096	0.396	0.24	0.249	0.025	0.167	0.165
8	0.129	0.222	0.58	0.194	0.056	0.114	0.095

Table III Results of X-ray Diffraction Investigations

Sample Symbol	Degree of Crystallization	Average Lateral Crystallite Size (Å)		
		D (010)	D (110)	D (100)
1	0.41	79	—	114
2	0.40	69	—	114
3	0.38	66	—	114
4	0.39	66	—	99
5	0.16	—	167	97
6	0.18	—	187	94
7	0.16	—	227	100
8	0.15	—	288	114

The contribution of OH groups was examined on the basis of measured extinction values of 1320 cm^{-1} absorption bands (Table II). The extinction values of this band were always markedly smaller for membranes with 60% deacetylation than for membranes with 90% deacetylation. In light of these findings, it can justifiably be expected that chitosan membranes made of higher deacetylated chitin should exhibit higher chemical reactivity and, therefore, also bioactivity than those made of a lesser degree of deacetylated chitin.

CONCLUSIONS

The type of crystalline structure of chitosan membranes is determined by the degree of deacetylation of chitin utilized on manufacturing of the membrane. The processing technique of membranes (i.e., either the kind of chitosan solvent, HCOOH or CH_3COOH, or the kind of successively applied regenerating bath, $NH_4OH + C_2H_5OH$ or $NaOH + C_2H_5OH$) does not influence the type of the crystal structure established.

The established crystalline structure is characterized by a low degree of lattice perfection. The investigations carried out do not give evidence for the appearance of spherilitic crystalline aggregations.

The degree of crystallinity and the average lateral crystallite size basically depend on the degree of chitin deacetylation. In the case of lower deacetylation (60%), the degree of crystallinity is larger; the average lateral crystallite size is also larger than in the case of membranes characterized by a lower degree of deacetylation. The membrane preparation technique, i.e., the kind of chitosan solvent used and the kind of regenerating bath, only slightly influence the crystallization of the membranes.

The ascertained differences in the degree of crystallinity for membranes made of 60% and 90% deacetylated chitin leads to the conclusion that the second kind of membranes, as less crystalline, should exhibit higher bioactivity than should the first kind.

The larger contribution of accessible OH groups in membranes made of stronger deacetylated chitin corroborates the supposition about the higher chemical reactivity and higher bioactivity of such kinds of membranes.

Membranes with a lower degree of deacetylation exhibit at microscopic examination a more homogeneous morphological composition. Membranes made of 90% deacetylated chitin reveal a nonhomogenous, grainy morphological structure. It can be supposed that such a morphology may be in favor of the higher chemical reactivity and bioactivity of membranes made of a stronger deacetylated chitin.

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