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Studies of the Structure of Polysaccharides in the Process of Alkaline Treatment of Dibutyrylchitin Fibres

Abstract
Chitin, i.e. poly-(1-4)-2-acetamine-2-acetamine-2-deoxy-D-glucopyranose, is a natural polymer formed in the biosynthesis process. Like cellulose, it is a polysaccharide characterised by the ring structure of the chain links. In addition, chitin has a high molecular weight and a high degree of crystallinity. However, chitin is characterised by low solubility which considerably limits its processing, application and usability [1]. Thus, many efforts have been made to modify chitin by chemical treatment in order to obtain its derivatives (mainly dibutyrylchitin or chitosan) with improved solubility, while at the same time preserving its increased biological activity. Dibutyrylchitin fibres were manufactured from a solution of the polymer in ethyl alcohol by extrusion. The fibres obtained were then treated with an alkaline solution, namely aq. potassium hydroxide. By applying various parameters in the alkaline treatment, DBCH fibres can be transformed into fibres from regenerated chitin, or even into chitosan fibres. The chemical and structural changes occurring in the successive stages of the treatment were examined by means of wide-angle X-ray diffraction (WAXS). The investigation we carried out led to the final conclusions describing the structural changes occurring in the examined material.

Key words: *DBCH, supermolecular structure, WAXS.*

Introduction

Chitin, a well-known polymer abundant in nature, is characterised by extremely low solubility in organic solvents, which reduces its usability. On the other hand, one chitin derivatives, dibutyrylchitin (DBCH), is soluble in many popular solvents, and so its processing is much easier. Dibutyrylchitin was obtained from native krill chitin by its estrefication with butyric anhydride. From DBCH it is possible to obtain films, fibres, microspheres etc. [2,3].

Materials

In this study, dibutyrylchitin fibres formed from a polymer solution prepared in anhydrous ethyl alcohol were the subject of the investigation. The use of such a solvent made it possible to replace the acetone or dimethylformamide [4] applied so far, and to eliminate their toxic action.

The fibres were spun using a wet-dry method by introducing a partly solidified polymer stream into the air, and then into a water bath in which coagulation of DBCH took place and the fibres were double-stretched [5]. Next, the fibres were subjected to the successive stages of alkaline treatment. In the first stage when DBCH is transformed into chitin, the number of butyryl groups gradually decreases. In the second, from chitin to chitosan, the number of acetyl group lowers also.

This procedure was used for all the spinning solutions prepared, and yielded DBCH fibres of differentiated linear densities and various diameters 35, 50, 55, 67 and 75 mm. The SEM photographs of the surface and cross-sections of DBCH fibres are shown in Figure 1 and Figure 2.

Experimental

In the next stage of our investigation, DBCH fibres were subjected to the alkaline treatment. The dibutyrylchitin process was carried out using 5% solutions of potassium hydroxide at temperatures ranging from 20°C to 90°C.

The SEM photographs of the surface and cross-sections of regenerated chitin fibres are shown in Figure 3 and Figure 4.

The N-deacetylation process in the second stage was performed for the fibres from regenerated chitin and the initial DBCH fibres. For all the samples, the N-deacetylation process was carried out in saturated solutions of KOH at temperatures ranging from 70°C to 140°C.

The SEM photographs of the surface of chitosan fibres are shown in Figure 5 - Figure 8.

The modification of the dibutyrylchitin fibres obtained and fibres made of chitin regenerated to chitosan fibres was carried out using saturated solutions of potassium hydroxide.

The chemical and structural changes occurring in the successive stages of the treatment were examined by means of wide-angle X-ray diffraction (WAXS). The obtained diffractograms were analysed using the Hindeleh & Johnson method [6] and the Optifit computer program [9,10] which applied the Rosenbrock method [7] in the minimisation process. For the samples revealing crystalline peaks, interplanar distances were determined, as were the corresponding crystalline areas in the perpendicular direction to these planes [8].

Results and discussion

DBCH fibres treated by alkali at both the stage of chitin regeneration and later during the formation of chitosan undergo a chemical transformation, i.e. their side groups successively break off.

The wide-angle diffraction measurements of X-ray (WAXS) show an ordered structure and a high degree of crystallinity of the native krill chitin. The esterification of chitin into dibutyrylchitin results in distinct structural changes in the polymer obtained.

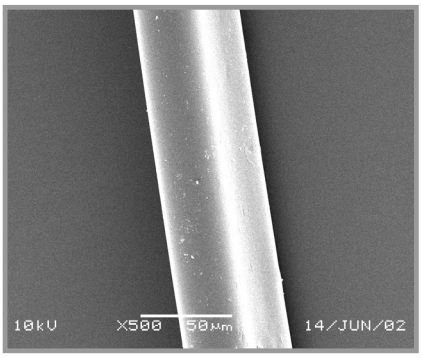

Figure 1. *SEM micrograph of the surface of DBCH fibres, magnification ×500.*

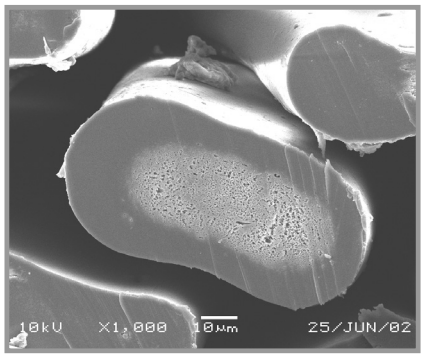

Figure 2. *SEM micrograph of the cross-sections of DBCH fibres, magnification ×1000.*

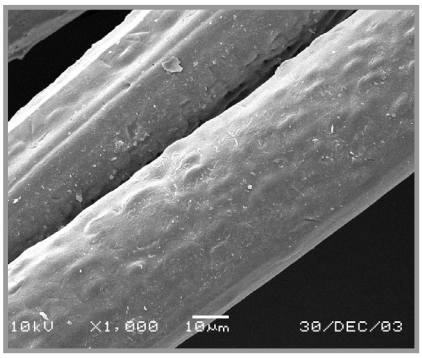

Figure 3. *SEM micrograph of the surface of regenerated chitin fibres, magnification ×1000.*

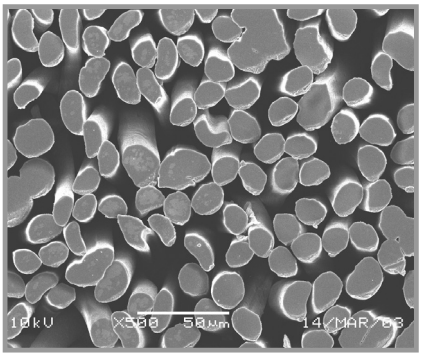

Figure 4. *SEM micrograph of the cross-sections of regenerated chitin fibres, magnification ×500.*

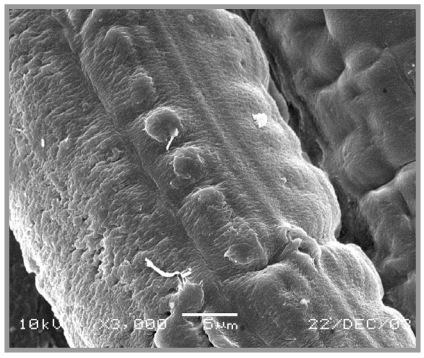

Figure 5. *SEM micrograph of the surface of chitosan fibres, series NC (70°C), magnification ×3000.*

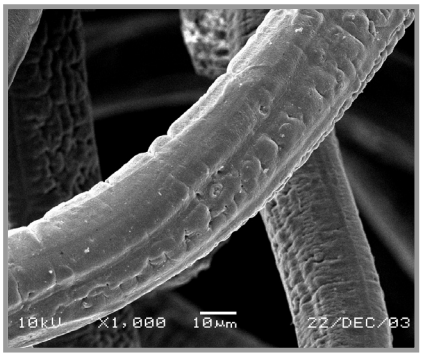

Figure 6. *SEM micrograph of the surface of chitosan fibres, series NC (70°C), magnification ×1000.*

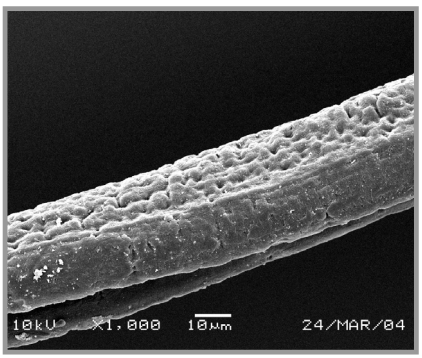

Figure 7. *SEM micrograph of the surface of chitosan fibres, series NG (120°C), magnification ×1000.*

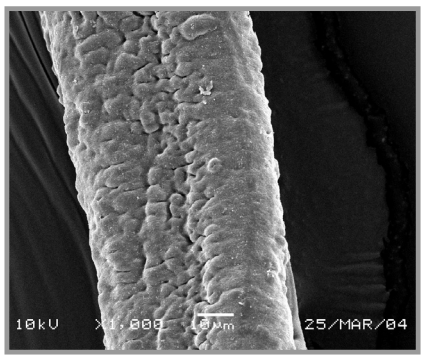

Figure 8. *SEM micrograph of the surface of chitosan fibres, series NH (140°C), magnification ×1000.*

During the deesterification process, a supermolecular structure is gradually formed in which the system of macromolecules becomes similar to the crystalline structure of the krill chitin. After 120 min. of the reaction carried out at 20°C, the WAXS diffractogram of the regenerated chitin fibres closely corresponds to the diffractogram obtained for the native krill chitin. At higher temperatures, the deestrification reaction proceeds much more quickly.

At 90°C, after 20 minutes of the reaction, the ester groups have already disappeared, and are not present in the spectroscopic results.

In the A, B, C and D deesterification series, regenerated chitin is the final product of hydrolysis, as it has a deesterification degree of zero. Its degree of crystallinity approaches the value obtained for the initial krill chitin.

The sizes of the ordered areas calculated on the basis of the half-width of crystalline reflexes do not reach the sizes characterising the initial krill chitin in any series, and only in the A series do they reach 70% of this value.

The gradual breaking-off of the butyric substituents which takes place during the hydrolysis probably causes the stabilisation of crystalline network defects in the regenerated chitin and brings about the widening of reflexes. As a result, the calculated sizes of crystallites are smaller than the corresponding ones for the native krill chitin.

The alkaline treatment does not significantly affect the localisation changes of the reflex (100) when $2\Theta=20^{o}$. The largest changes during the deesterification are observed for the reflex (010). The interplanar distance calculated on the basic of the above reflex gradually approaches the corresponding distance for the krill chitin in the successive stages of the alkaline treatment. (Table 1, and Figures 9-11).

In DBCH fibres, no other reflexes are observed. In the deesterification series A, B, C and D only, the period of reaching the interplanar distance in the perpendicular direction (010) becomes shorter, which makes the polymer similar to the krill chitin. The remaining interplanar distances directed perpendicularly (220), (100), (110) in all four series (A, B, C, and D) reach the values characteristic of the krill chitin.

N-deacetylation of the regenerated chitin is a process which again causes the destruction of the ordering which is characteristic of chitin macromolecules. A gradual decrease in the number of N-acetyl groups and the growth of NH_2 groups number affects the molecular interaction and the force of hydrogen bonds. As in the debutyration process, the deformation effect of the tactic structure of the molecular chain appears again. Then its successive comeback, observed earlier in the case of the DBCH fibres, takes place. However, this process does not lead to the total removal of acetyl groups. The remaining substituents cause significant disturbances in the spatial system of macromolecules. When the polymer structure is investigated using the WAXS method, the above phenomenon is observed as almost full amorphisation of the polymer.

Table 1. *Results of WAXS examination for krill chitin, DBCH and products of DBCH alkaline hydrolysis of 20°C.*

Sample	Time alkaline treatment at 20°C	Degree of crystallinity	Crystallite size				Interplanar distance			
			$D_{(010)}$	$D_{(220)}$	$D_{(100)}$	$D_{(110)}$	$d_{(010)}$	$d_{(220)}$	$d_{(100)}$	$d_{(110)}$
	min	%	nm	nm	nm	nm	nm	nm	nm	nm
DBCH	-	32	2.0	-	0.91	-	1.2	-	0.43	-
A1	10	37	2.2	-	1.4	-	1.2	-	0.43	-
A2	20	37	2.1	-	1.4	-	1.17	-	0.43	-
A3	30	44	1.9	3.2	2.7	-	1.08	0.66	0.46	-
A4	60	47	4.1	5.9	4.7	5.2	0.98	0.69	0.46	0.34
A5	120	62	4.2	7.5	6.8	4.2	0.99	0.70	0.46	0.34
A6	240	72	4.7	5.8	5.2	4.8	0.98	0.69	0.46	0.34
A7	480	74	4.5	4.9	4.6	4.0	0.98	0.69	0.46	0.34
A8	960	77	4.7	4.3	4.6	4.9	0.99	0.69	0.46	0.34
Krill chitin	-	78	6.9	7.8	5.2	6.3	0.96	0.69	0.46	0.34

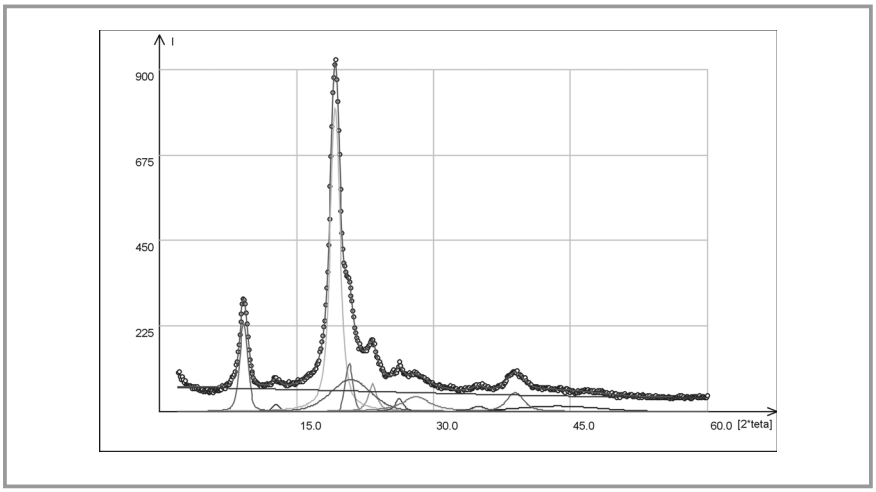

Figure 9. *Peak deconvolution of the WAXS profile in the initial krill chitin.*

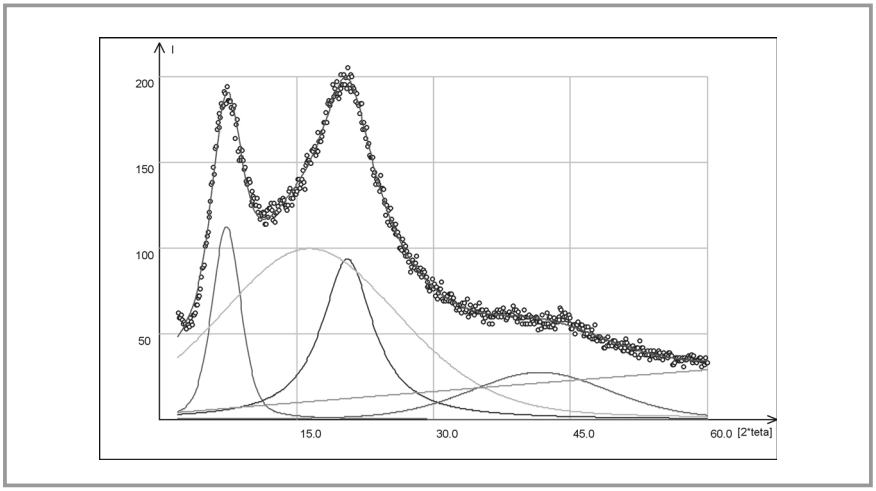

Figure 10. *Peak deconvolution of the WAXS profile in the DBCH fibres.*

The intermolecular distances characteristic for chitin undergo deformation, which causes the disappearance of crystalline reflexes. Thus, the N-deacetylation process brings about the amorphisation of the polymer.

Amorphisation of the polymer is probably affected by the non-statistic course of the deacetylation process. The remaining side groups cause local formation of hydrogen bonds, which preserves the defects of the spatial system of macromolecules.

At first N-deacetylation causes a significant lowering (up to 3 times in comparison with the initial material) of the degree of crystallinity. The longer duration of the process in the N C series (70°C) or the high deacetylation degree in the N H series (140°C) bring about the increase in crystallinity. For the reflex observed, the size of crystallites was calculated and the interplanar distance in the perpendicular direction (100) (Table 2, Figure 12) was determined.

Conclusions

- The alkaline treatment of DBCH fibres results in the reduced amounts of the butyryl groups and the acetyl group.
- In each deesterification series, the calculated degree of crystallinity is close to that of krill chitin.
- The molecular structure of the regenerated chitin obtained, as examined by means of WAXS, corresponds to the structure of the initial krill chitin.
- When the polymer structure is investigated using the WAXS method, the above phenomenon is observed as almost full amorphisation of the polymer.

References

1. *R.A.A. Muzzarelli, C. Muzzarelli, M. Terbojevich, Carbohydr. in Europe, 19, 10-18 1997.*
2. *L. Szosland, 'Di-O-butyrylchitin' in Chitin Handbook, R.A.A. Muzzarelli, M.G. Peter (eds.) 1997, 53-60.*

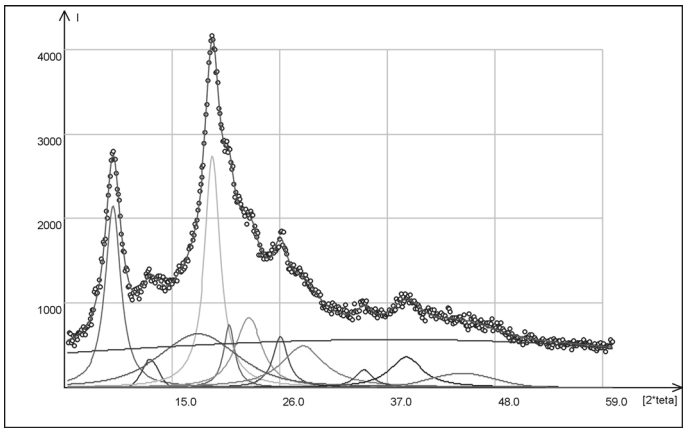

Figure 11. *Peak deconvolution of the WAXS profile in the regenerated chitin fibres.*

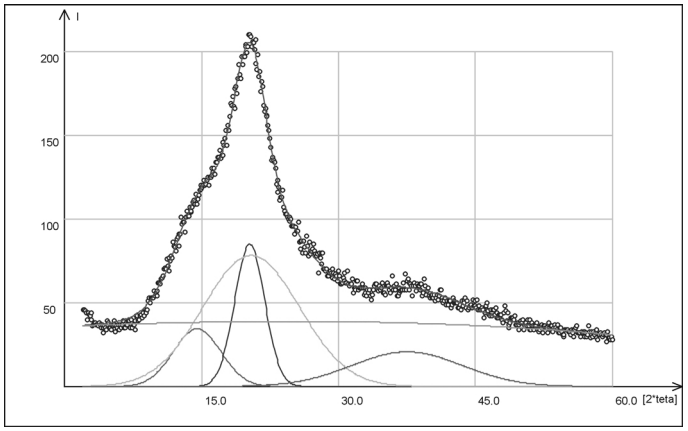

Figure 12. *Peak deconvolution of the WAXS profile in the chitosan fibres.*

Table 2. *Results of WAXS examination for regenerated chitin and products of regenerated chitin to obtain N-deacetylation of 140°C.*

Sample	N-deacetylation at 140°C	Degree of crystallinity	Crystallite size				Interplanar distance			
			$D_{(010)}$	$D_{(220)}$	$D_{(100)}$	$D_{(110)}$	$d_{(010)}$	$d_{(220)}$	$d_{(100)}$	$d_{(110)}$
	min	%	nm	nm	nm	nm	nm	nm	nm	nm
Regenerated chitin	180	74	3.8	4.9	4.7	8.0	0.98	0.69	0.46	0.34
N H1	10	24	-	-	2.1	-	-	-	0.45	-
N H3	30	21	-	-	2.3	-	-	-	0.45	-
N H4	60	21	-	-	2.3	-	-	-	0.44	-
N H5	120	24	-	-	2.3	-	-	-	0.44	-
N H6	180	27	-	-	2.3	-	-	-	0.44	-
N H7	240	30	-	-	2.3	-	-	-	0.44	-

3. *L. Szosland, 'Synthesis of Highly Substituted Butyryl Chitin in the Presence of Perchloric Acid', J.Bioactive and Compatible Pol., 11, 61-71, 1996.*
4. *L. Szosland, G.C. East, 'The dry spinning of dibutyrylchitin fibers', J.Appl. Pol. Sci., 58, 2459-2466, 1995.*
5. *W. Biniaś, A. Włochowicz, D. Biniaś, S. Boryniec, Patent nr P359883.*
6. *A.M. Hindeleh, D.J. Johnson: J. Phys. D: Appl. Phys., 4, 259, 1971.*
7. *H.H. Rosenbrock, C. Storey, Computational Techniques for Chemical Engineers, Pergamon Press, 1966.*
8. *L.E. Alexander, 'X-ray Diffraction Methods in Polymer Science'; Wiley New York 1969.*
9. *M. Rabiej, Polimery 2002, 47, 6, 423.*
10. *M. Rabiej, Polimery 2003, 48, 4, 288.*

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