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Chitosan Microfibrils: Preparation, Selected Properties and Application

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Abstract

According to a method elaborated in the Institute of Biopolymers and Chemical Fibres (the former Institute of Chemical Fibres), Łódź, Poland, we prepared chitosan microfibrils with a diameter of 1 to 5 μm in wet condition, which shrinks to the range of only 0.2 to 1.0 μm after drying. We determined the optimum properties of the spinning solutions, suitable for the successful formation of microfibrils. We studied how the forming conditions, such as the flow speed of the spinning solution and the coagulation bath, influence the properties of the formed microfibrils, principally dimensions and water retention value. Investigations into the molecular, super-molecular and morphological structure of the microfibrils provided much information on the characteristics of this new form of chitosan. We also evaluated the possibility of using microfibrils for the preparation of nonwovens.

Key word: microfibrils, wet spinning, chitosan microfibrils' properties.

Introduction

Chitosan, poly-(1-4)- β -glucosamine, constitutes a valuable raw material in the production of film, fibres, beads, sponges, microcrystalline chitosan and fibrils [1-3]. Presently, chitosan is used in medicine, veterinary, husbandry, cosmetics and the food industry [1]. The fibrils are formed hydrodynamically from a solution of the polymer, and are characterised by a large developed surface. The shape of the fibrils depends upon the quality of the polymer, the composition of the coagulation bath and the forming conditions. Fibrils may appear as short, very fine fibres or small fragments of film [4]. Various polymers can be used in the preparation of fibrils, depending up their expected properties and destination.

Fibrils made of cellulose derivatives have long been produced commercially by many very well-known companies [5, 6]. The manufacturing methods usually employ organic solvents. Processes have also been described in which novel equipment is applied, allowing for a substantial saving of solvent [7]. In the Institute of Biopolymers and Chemical Fibres (the former Institute of Chemical Fibres), investigations are carried out aimed at developing new methods to produce microfibrils from several materials including cellulose, cellulose carbamate, starch, alginates, chitosan and blends such as alginate/starch and alginate/keratin [4, 8].

The fibrils' properties can be modified by blending various polymer solutions during formation [8]. Nonwovens manufactured from chitosan fibrils and fibres of carboxymethylcellulose are characterised by bioactive properties which enable their use in medicine [9]. Fibrils of cellulose

acetate, due to their developed internal surface, lend themselves to use not only as filter material but also in the purification of effluents, albumin binding, and as agents accelerating the sedimentation of impurities [10-14]. Applications are also reported [15] are also of fibrils with extensively developed surfaces reaching the range of 50 to even 100 m^2/g of the polymer.

The preparation of microfibrils requires specific equipment that is capable of providing high shearing forces in the coagulation zone of the polymer.

In the Institute of Biopolymers and Chemical Fibres, a prototype equipment unit was constructed for the wet forming of microfibrils from solutions of natural polymers. By means of this device, microfibrils can be formed within the diameter range of 1 to 5 μm in wet conditions. It is an advantage of the apparatus that organic solvents are not used in the forming process, but instead, aqueous solutions of chitosan salts as well as aqueous coagulation baths at low concentration.

The objective of this work was to elaborate the process parameters for forming chitosan microfibrils using the prototype, as well as to estimate the properties of the products obtained including the super-molecular and morphological structure.

Materials used

The following kind of chitosan was used in the work: Primex III-90 from Primex BioChemicals AS, Norway featuring a deacetylation degree of 82.2% and a viscometric average molecular weight $M_v = 346.0 \text{ kD}$.

Research methods

Preparation of chitosan solution

The chitosan solution is prepared in a mixer equipped with a fast stirrer. The chitosan is added to water at 20 $^{\circ}\text{C}$, then aqueous acetic or hydrochloric acid is poured in at constant agitation. The concentration of the acids was 3 wt.% for acetic and 0.4 wt.% for hydrochloric acid. The solution obtained is filtered through a frame filter press using a polypropylene filter cloth to withhold impurities larger than 1 μm . The chitosan solution was next diluted to a 1.5% concentration of acetic acid in the spinning bath.

Preparation of microfibrils

The fibrils are formed according to the method described elsewhere [8]. The method involves the polymer solution streaming out of the spinneret and being snatched away by the coagulation baths which stream around the spinneret at high speed, thus pulling and breaking the microfibrils instantly during their forming. The bath containing microfibrils is continuously collected in a tank. The microfibrils are then separated from the bath and washed with water. Aqueous sodium hydroxide is used as the coagulation bath.

Preparation of nonwoven from the microfibrils

The preparation of the nonwoven was accomplished by introducing a sufficient amount of ethyl alcohol to a water suspension of the microfibrils to obtain a 20% solution. The suspension of chitosan microfibrils is eventually freeze-dried for 8 hours.

■ Analytical methods

Determining the chitosan content in the solution

The method consists in estimating the content of chitosan in the solution after its regeneration in the coagulation bath. About 10 ± 0.0002 g of chitosan is placed on a dry Teflon plate. The sample is covered with another plate and evenly distributed between the plates by delicately pressing the two plates together. The plates are cautiously pulled away from each other and dried at 80 °C, and then immersed in an aqueous coagulation bath containing 5% of NaOH at 20 °C. The small films obtained are next rinsed carefully with distilled water. The surplus of water is squeezed away from the films, which are put into a weighing glass and dried to a constant temperature. The percentage content of chitosan (X) is calculated from the following formula:

$$X = m_2/m_1 \times 100, \%$$

where:

m_1 - weight of chitosan solution, g

m_2 - weight of the dry chitosan film g

Determining the dynamic viscosity of the chitosan solution

The dynamic viscosity of the chitosan solution is determined with the use of a Brookfield viscometer, type LVR (serial number 112285)

Determining the corrected clogging value K_w^* of the chitosan spinning solution

The description can be found elsewhere [8].

Analysing the molecular weight distribution in chitosan and chitosan microfibrils by gel chromatography (GPC)

In the GPC analysis, we used a gel chromatograph equipped with a Hewlett-Packard 1050 pump and a Hewlett-Packard 1047 refractometric detector. The analysis was carried out according to methods described elsewhere [16, 17].

Determining the crystallinity of chitosan and the microfibrils thereof

The wide-angle X-ray investigations (WAXS) were done with the use of the HZG-4 diffractometer (Seifert, Germany) in the University in Bielsko-Biala, Poland according to the Hindeleh & Johnson method [18]. The diffractograms were processed with the aid of a computer programme applying Rosenbrock's method in the minimisation [19].

Microscopic inspection of the chitosan microfibrils

The dimensions of the chitosan microfibrils were estimated by means of a Biolar polarising microscope (ZPO Warsaw, Poland) with a computer image analyser. The microscopic photos of the dry microfibrils and the nonwoven made from them were taken with the use of a JEOL 35C scanning electron microscope at magnification $\times 5000$.

Determining the length of chitosan microfibrils by means of the ADV fibre length analyser

The length of the chitosan microfibrils was measured using a ADV-3 length analyser (VUPC Bratislava, Slovakia). The method consists in measuring the microfibrils' length in fibrous form streaming in a diluted suspension. The measuring element is a conductivity detector fixed in a capillary, 0.35 mm in diameter. The analysis was carried out in the Pulp and Paper Institute in Łódź, Poland.

Determining the water retention value (WRV) of chitosan microfibrils

This is given elsewhere [8].

■ Results of the investigations

Investigations in the process of preparing chitosan microfibrils

The chitosan microfibrils were formed from aqueous chitosan solutions prepared with either acetate or hydrochloric

acid. The properties of the microfibrils and prepared nonwovens depend upon the kind of acid used in the process. Initially, the investigations were carried out with aqueous solutions of chitosan in acetic acid. Based on our own experience, two spinning solutions were prepared: one with the concentration of chitosan amounting to 4.4% and acetic acid to 3%, and 2.2% of chitosan and 1.5% of acetic acid in the other. The conditions under which the microfibrils were prepared can be seen in Table 1. We studied the impact of the chitosan concentration in the spinning solution on the microfibrils formed. At a higher chitosan concentration in the spinning solution, the dimensions of the fibrils largely diversified. Microfibrils spun from acetic acid solutions are susceptible to mechanical and chemical deformation in the course of washing, during which short microfibrils fragments are formed, as are particles resembling microcrystalline chitosan. The finer the microfibrils, the easier they can be damaged. This was why further investigation in the spinning from chitosan solutions in acetic acid was abandoned. The first attempt to form microfibrils from chitosan solution at its concentration of 0.5% in 0.2% hydrochloric acid (F-81) revealed a positive change in the product's properties. The low concentrations of both the polymer and solvent resulted in the improved uniformity of the chitosan microfibrils. No defects occurred during the washing. The spinning trials were running smoothly, and the obtained product was homogeneous. The fibrils' lengths ranged between 500 and 800 μm . In Figure 1, microscopic images of chitosan microfibrils in wet condition can be seen, marked F-81. The length was measured with the use of an optical microscope. Measurements were also made with a fibre length analyser for comparison. In Figure 2, the distribution of the length of chitosan microfibrils marked F-81 is presented. The calculated arithmetical average length of

Table 1. Conditions for manufacture and selected properties of chitosan microfibrils; V_r : V_k - proportion of flow speed of spinning solution to flow speed of coagulation bath.

Microfibril's symbol	Solvent	Concentration in spinning solution, wt%		Dynamic viscosity at 20 °C, cP	Kw*	Concentration of NaOH in coagulation bath, g/l	Vr : Vk	Content of microfibrils in water suspension, wt%	WRV, %	Range of average size of microfibrils, μm	
		polymer	solvent							diameter	length
F - 61	CH ₃ COOH	4.40	3.0	3900	145.0	20.0	1:10	3.59	515	2 - 20	500 - 900
F - 69		2.20	1.5	2650	0	10.0	1:17	3.45	491	1 - 10	300 - 800
F - 81	HCl	0.50	0.2	17	88.0	10.0	1:10	0.46	573	1 - 3	500 - 800
F - 109							1:83	0.41	594	1 - 3	150 - 300
F - 110							1:165	0.32	615	1 - 2	100 - 300

the microfibrils amounts to 1.09 mm, and is close to that determined by the use of microscope. On the basis of the results presented in Table 1, it can be stated that the dimensions of the chitosan microfibrils are also influenced by the speed proportion of the outflow of the spinning solution and the streaming coagulation bath. With the increasing speed of the coagulation bath, the chitosan microfibrils become finer and much shorter.

Molecular weight distribution in chitosan and microfibrils

Samples of Primex III chitosan and microfibrils F-81 and F-110 were selected for the investigations. The same microfibrils were next used in the preparation of the nonwoven. The microfibrils were formed from aqueous hydrochloric acid which, apart from being the chitosan solvent, may also cause its degradation depending upon the dissolving conditions. Table 2 presents the investigation results of the molecular weight distribution of chitosan and the microfibrils produced from it.

On the basis of the results presented in Table 2, it was found that the weight average molecular weight M_w of the chitosan microfibrils marked as F-81 amounts to 110.4 kD, and compared to the initial chitosan, is lower by 46 kD. The decrease is a result of the polymer degrading during dissolving. A trial, in which the chitosan microfibrils F-110 were formed at a coagulation bath speed higher by a dozen times or so, confirmed that under such conditions finer and shorter microfibrils are formed with a similar molecular weight. This is good evidence of the process' repeatability. In Figure 3, the function of molecular weight distribution is presented for the initial chitosan Primex III, and the microfibrils F-81 and F-110.

X-ray investigations of chitosan and microfibrils

Table 3 presents the results of investigations carried out on the initial chitosan and microfibrils made from it.

From the data presented in Table 3, it can be seen that the crystallinity degree of the initial chitosan Primex III is higher than that of the prepared microfibrils, which is lower by about 10%. The lower value of the microfibrils' crystallinity may result from the poor orientation of the macromolecules and the lack of fixation of the

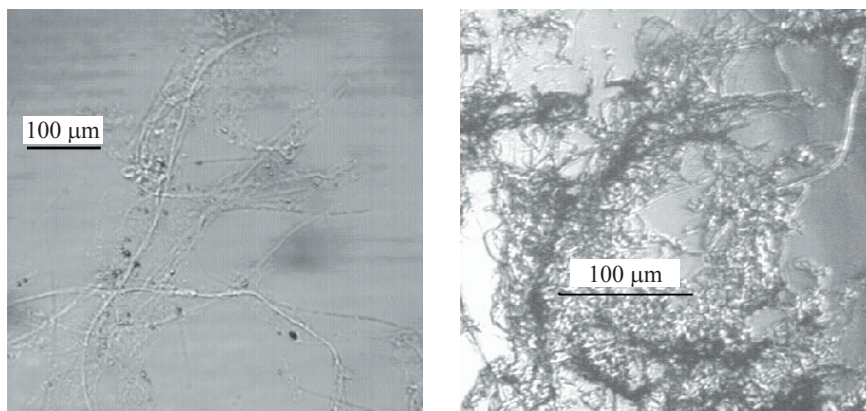


Figure 1. Microscope photos of chitosan microfibrils F-81 in wet conditions.

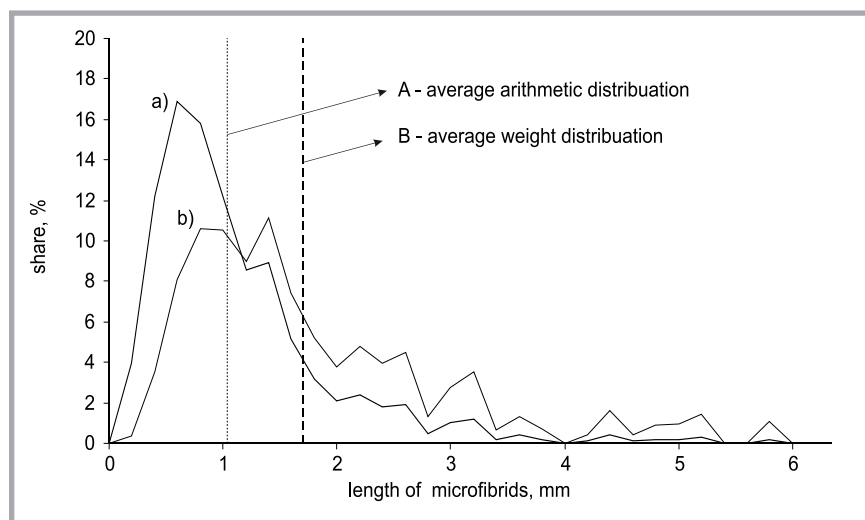


Figure 2. Length distribution of chitosan microfibrils (F-81) drawn with the use of the length analyser; a – arithmetic distribution, b – weight distribution.

Table 2. Molecular characteristic of the initial chitosan Primex III and microfibrils thereof; Pd - polydispersity.

Trial symbol	Mn, kD	Mw, kD	Pd	Percentage $M \times 10^{-3}$, D						
				< 5	5 – 50	50 – 100	100 – 200	200 – 400	400 – 800	>800
Primex III	48.6	156.4	3.22	1	28	25	24	15	5	2
F-81	45.6	110.4	2.42	-	30	29	27	11	3	-
F-110	46.5	109.6	2.35	1	31	29	26	11	2	-

Table 3. Values of the crystallinity degree and crystallite size, determined by the method of wide-angle x-ray spreading (WAXS).

Trial symbol	Crystallinity degree, %	Size of crystallites, Å		
		D ₁	D ₂	D ₃
Primex III	35.0	26.1	62.2	50.7
Chitosan microfibriles F-110	24.6	22.0	49.6	52.6

formed structure which occurs in the standard spinning process of fibres. The process of preparing microfibrils, as presented here, offers products of low crystallinity.

In Figure 4, the diffraction patterns of Primex III and the microfibrils are shown.

Estimating the microfibrils by means of scanning electron microscopy (SEM)

The size of the microfibrils formed was measured using an optical microscope. The original shape of these microfibrils can be preserved by keeping them in a water suspension. Washing, concentrating and drying may easily cause changes

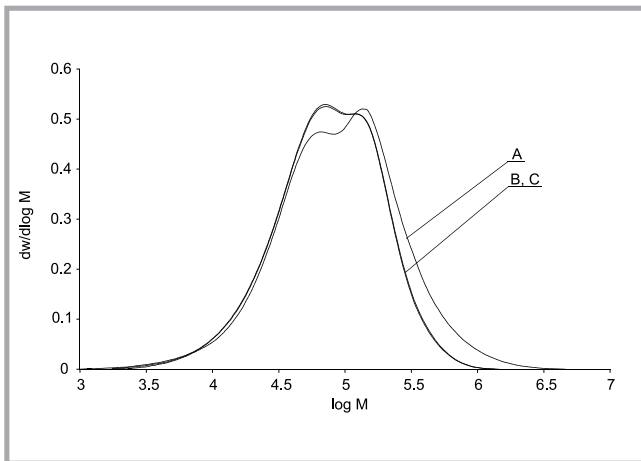


Figure 3. Distribution of molecular weight for: A - initial chitosan Primex III, B - chitosan microfibrils F-81, C - chitosan microfibrils F-110, determined by gel chromatography.

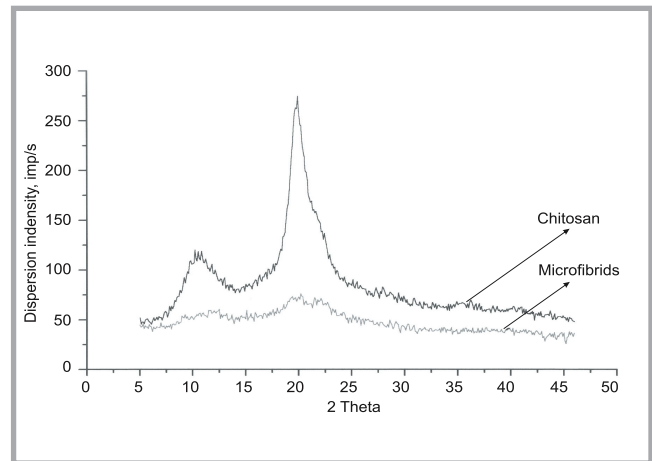


Figure 4. Diffraction patterns of Primex III chitosan and microfibrils F-110, determined by WAXS investigation, with the use of HZG-4 diffractometer.

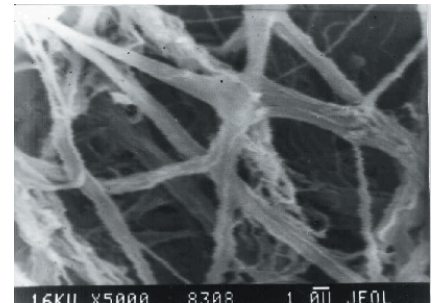
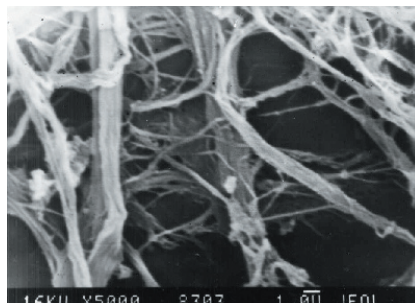
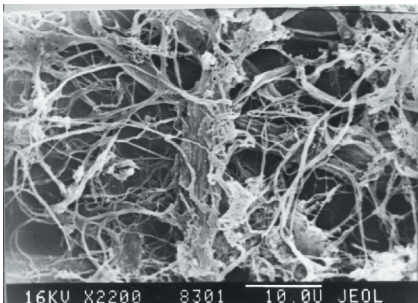


Figure 5. SEM photos of chitosan microfibrils marked F-81, in dry conditions; diameter into the range of 0.2 - 1.0 μm .

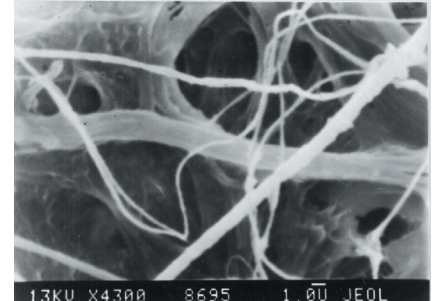
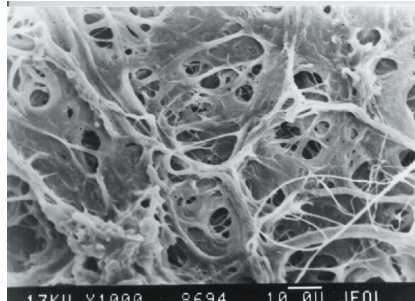
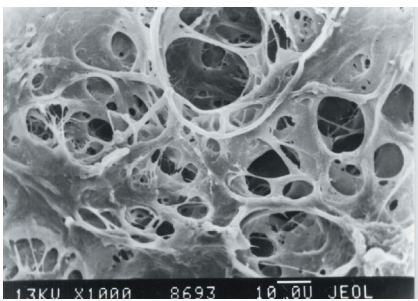


Figure 6. SEM photos of the surface of a nonwoven made of chitosan microfibrils, marked F-110, freeze-dried.

of the shape, in particular the length. For taking SEM photos, the microfibrils must be dried. First, the water was removed by multiple washing with acetone and ethyl alcohol, followed by drying at ambient temperature. The SEM pictures of the chitosan microfibrils are presented in Figure 5. They show microfibrils in dry conditions with a diameter several times smaller than those of wet material. The diameter of elementary microfibrils falls into the range of 0.2 to 1.0 μm .

Investigating the preparation of non-wovens from the microfibrils

The preparation of nonwovens from the microfibrils from their water suspension

is not easy, as they reveal an extensive tendency to stick together. This can, albeit only to a certain degree, be prevented by adding some anti-stick agents such as glycerol. Freeze-drying is an ever more frequently applied procedure in drying polymers from water suspensions. The method was used here since it provides a chance of preserving the polymer structure during drying. The first trial was begun by preparing the nonwoven in wet conditions, which was then freeze-dried. In the investigation, microfibrils marked F-81 were used. A nonwoven was obtained, built up by elemental microfibrils. Figure 6 presents SEM photos of the surface of a freeze-dried nonwoven

Conclusions

- On the basis of our investigations, it can be concluded that chitosan microfibrils can be prepared by forming them from diluted chitosan solutions in hydrochloric acid. The dimensions of the microfibrils are as follows: diameter in the range of 1 to 3 μm , length in the range of 100 to 300 μm in wet conditions.
- The parameters, dimensions and WRV of the microfibrils allow their application in sanitary nonwovens.
- The possibility was confirmed that a nonwoven can be prepared from chitosan microfibrils by freeze-drying.

- In future research, ways should be sought to improve the method of preparing nonwovens from chitosan microfibrils.



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