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Chitosan Microfibrids: 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 microfibrids 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 microfibrids. 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 microfibrids, principally dimensions and water retention value. Investigations into the molecular, super-molecular and morphological structure of the microfibrids provided much information on the characteristics of this new form of chitosan. We also evaluated the possibility of using microfibrids for the preparation of nonwovens.

Key word: *microfibrids, wet spinning, chitosan microfibrids' properties.*

Introduction

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

Fibrids 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 microfibrids from several materials including cellulose, cellulose carbamate, starch, alginates, chitosan and blends such as alginate/starch and alginate/keratin [4, 8].

The fibrids' properties can be modified by blending various polymer solutions during formation [8]. Nonwovens manufactured from chitosan fibrids and fibres of carboxymethylocellulose are characterised by bioactive properties which enable their use in medicine [9]. Fibrids 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 fibrids with extensively developed surfaces reaching the range of 50 to even 100 m^2/g of the polymer.

The preparation of microfibrids 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 microfibrids from solutions of natural polymers. By means of this device, microfibrids 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 microfibrids 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 Mv = 346.0 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 ˚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 1μm. The chitosan solution was next diluted to a 1.5% concentration of acetic acid in the spinning bath.

Preparation of microfibrids

The fibrids 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 microfibrids instantly during their forming. The bath containing microfibrids is continuously collected in a tank. The microfibrids are then separated from the bath and washed with water. Aqueous sodium hydroxide is used as the coagulation bath.

Preparation of nonwoven from the microfibrids

The preparation of the nonwoven was accomplished by introducing a sufficient amount of ethyl alcohol to a water suspension of the microfibrids to obtain a 20% solution. The suspension of chitosan microfibrids 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 microfibrids 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 microfibrids 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-Biała, 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 microfibrids

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

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

The length of the chitosan microfibrids was measured using a ADV-3 length analyser (VUPC Bratislava, Slovakia). The method consists in measuring the microfibrids' 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 microfibrids

This is given elsewhere [8].

Results of the investigations

Investigations in the process of preparing chitosan microfibrids

The chitosan microfibrids were formed from aqueous chitosan solutions prepared with either acetate or hydrochloric acid. The properties of the microfibrids 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 microfibrids were prepared can be seen in Table 1. We studied the impact of the chitosan concentration in the spinning solution on the microfibrids formed. At a higher chitosan concentration in the spinning solution, the dimensions of the fibrids largely diversified. Microfibrids spun from acetic acid solutions are susceptible to mechanical and chemical deformation in the course of washing, during which short microfibrids fragments are formed, as are particles resembling microcrystalline chitosan. The finer the microfibrids, 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 microfibrids 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 microfibrids. No defects occurred during the washing. The spinning trials were running smoothly, and the obtained product was homogeneous. The fibrids' lengths ranged between 500 and 800 μm. In Figure 1, microscopic images of chitosan microfibrids 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 microfibrids marked F-81 is presented. The calculated arithmetical average length of

Table 1. *Conditions for manufacture and selected properties of chitosan microfibrids; Vr : Vk - proportion of flow speed of spinning solution to flow speed of coagulation bath.*

Microfibrid'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 microfibrids in water suspension, wt%	WRV, %	Range of average size of microfibrids, μm	
		polymer	solvent							diameter	length
F - 61	CH_3COOH	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 microfibrids 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 microfibrids 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 microfibrids become finer and much shorter.

Molecular weight distribution in chitosan and microfibrids

Samples of Primex III chitosan and microfibrids F-81 and F-110 were selected for the investigations. The same microfibrids were next used in the preparation of the nonwoven. The microfibrids 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 microfibrids produced from it.

On the basis of the results presented in Table 2, it was found that the weight average molecular weight Mw of the chitosan microfibrids 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 microfibrids F-110 were formed at a coagulation bath speed higher by a dozen times or so, confirmed that under such conditions finer and shorter microfibrids 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 microfibrids F-81 and F-110.

X-ray investigations of chitosan and microfibrids

Table 3 presents the results of investigations carried out on the initial chitosan and microfibrids 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 microfibrids, which is lower by about 10%. The lower value of the microfibrids' crystallinity may result from the poor orientation of the macromolecules and the lack of fixation of the formed structure which occurs in the standard spinning process of fibres. The process of preparing microfibrids, as presented here, offers products of low crystallinity.

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

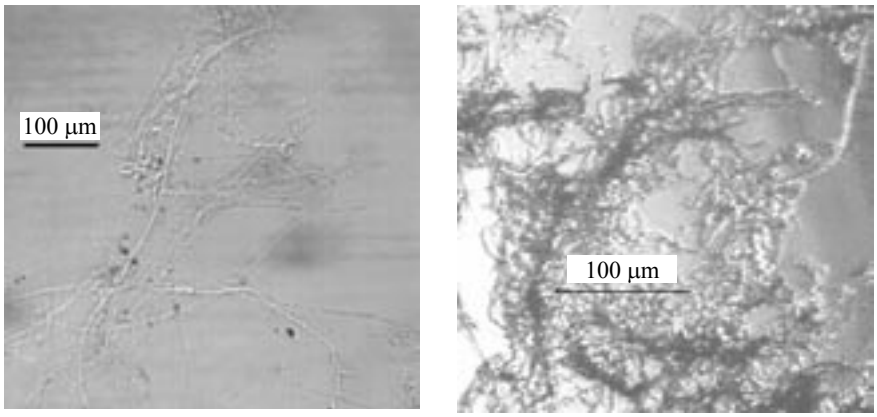

Figure 1. *Microscope photos of chitosan microfibrids F-81 in wet conditions.*

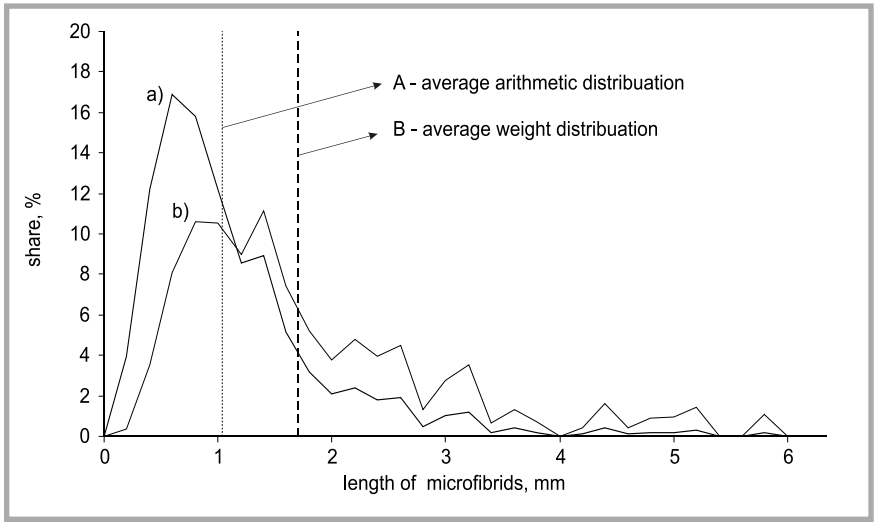

Figure 2. *Length distribution of chitosan microfibrids (F-81) drawn with the use of the length analyser; a – arithmetic distribution, b – weight distribution.*

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

Trial symbol	Mn, kD	Mw, kD	Pd	Percentage M × 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 microfibrides F-110	24.6	22.0	49.6	52.6

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

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

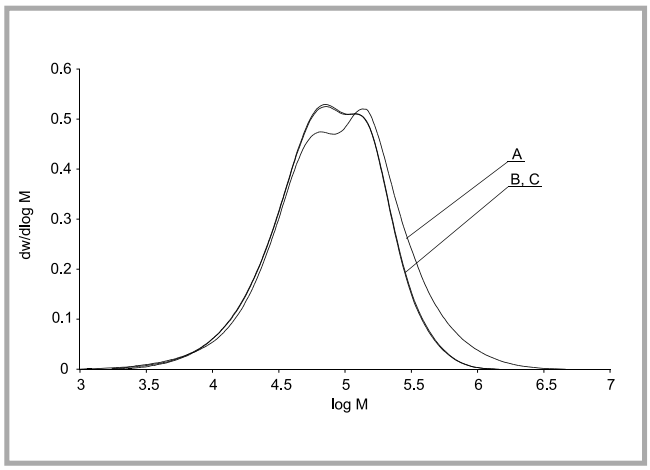

Figure 3. *Distribution of molecular weight for: A - initial chitosan Primex III, B – chitosan microfibrids F-81, C - chitosan microfibrids F-110, determined by gel chromatography.*

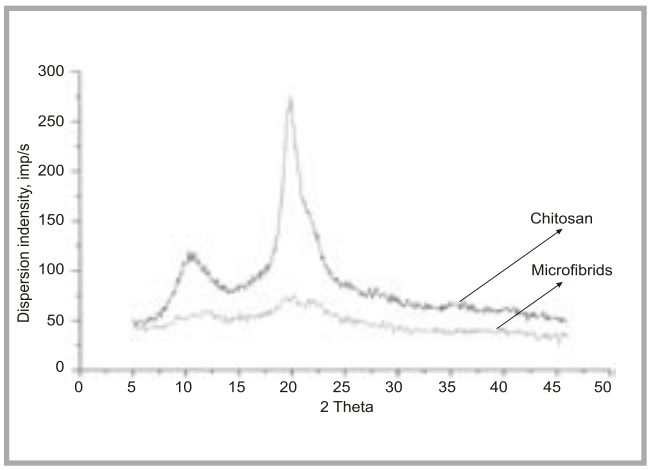

Figure 4. *Diffraction patterns of Primex III chitosan and microfibrids F-110, determined by WAXS investigation, with the use of HZG-4 diffractometer.*

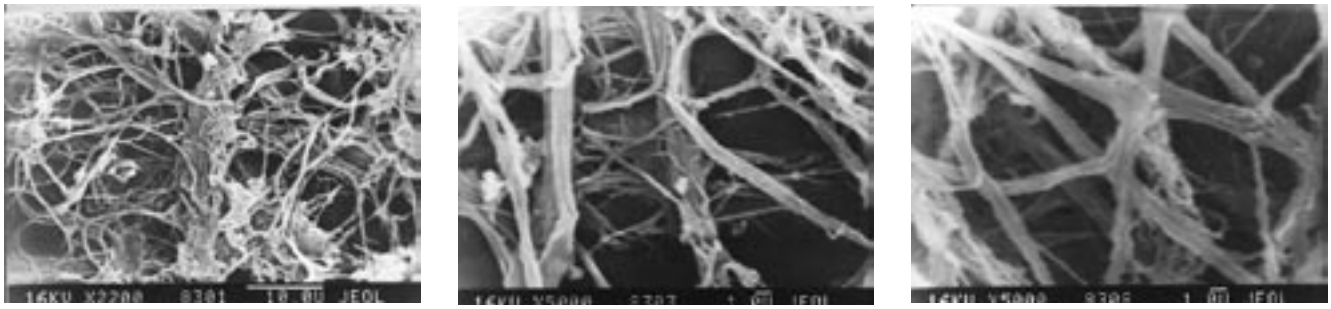

Figure 5. *SEM photos of chitosan microfibrids marked F-81, in dry conditions; diameter into the range of 0.2 - 1.0 μm.*

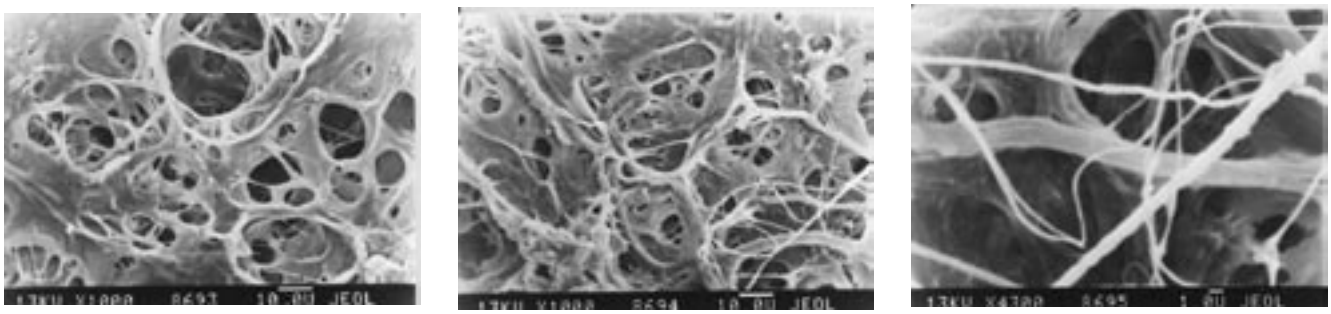

Figure 6. *SEM photos of the surface of a nonwoven made of chitosan microfibrids, marked F-110, freeze-dried.*

of the shape, in particular the length. For taking SEM photos, the microfibrids 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 microfibrids are presented in Figure 5. They show microfibrids in dry conditions with a diameter several times smaller than those of wet material. The diameter of elementary microfibrids falls into the range of 0.2 to 1.0 μm.

Investigating the preparation of nonwovens from the microfibrids

The preparation of nonwovens from the microfibrids 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, microfibrids marked F-81 were used. A nonwoven was obtained, built up by elemental microfibrids. 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 microfibrids can be prepared by forming them from diluted chitosan solutions in hydrochloric acid. The dimensions of the microfibrids are as follows: diameter in the range of 1 to 3 μm, length in the range of 100 to 300μm in wet conditions.
- The parameters, dimensions and WRV of the microfibrids allow their application in sanitary nonwovens.
- The possibility was confirmed that a nonwoven can be prepared from chitosan microfibrids by freeze-drying.

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

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Received 23.07.2006 Reviewed 27.08.2006