

# Microcrystalline Chitosan. I. Preparation and Properties of Microcrystalline Chitosan

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## Synopsis

A new type of microcrystalline polymer prepared by aggregation has been discussed. The effect of the parameters of the preparation process on the properties of microcrystalline chitosan in hydrogel and solid forms has been studied using also microscopy and spectroscopy. The product obtained according to the method discussed shows the average molecular weight within a range  $2.2\text{--}5.0 \times 10^5$ ,  $\text{WRV}_s \approx 260\text{--}520\%$ ,  $\text{WRV}_g \approx 700\text{--}1000\%$ , and CrI up to 95%.

## INTRODUCTION

Since microcrystalline cellulose has been discovered<sup>1</sup> some new microcrystalline polymers, both natural as well as synthetic, were investigated on theoretical and practical aspects.<sup>1-3</sup>

Methods of preparation of microcrystalline polymers consist, first of all, in (a) extraction of the nonordered regions from initial materials by both physical and chemical, or chemical processes alone, or (b) aggregation of macromolecules from solutions or dispersions via coagulation, precipitation, or crystallization.<sup>1-3</sup>

Microcrystalline chitin, described for the first time by Dunn et al.,<sup>4</sup> was prepared in a process designed for microcrystalline cellulose, the process consisting of a use of hydrochloric acid to remove the nonordered phase by hydrolysis and degradation. The use of 2-propanol solution of phosphoric acid in the hydrolysis process has proved to be more effective for preparation of microcrystalline chitin.<sup>5</sup>

Chitosan<sup>6</sup> (poly-D-glucosamine), prepared from chitin by its *N*-deacetylation at elevated temperature in the presence of alkalies, has not been sufficiently known as yet in the microcrystalline form.

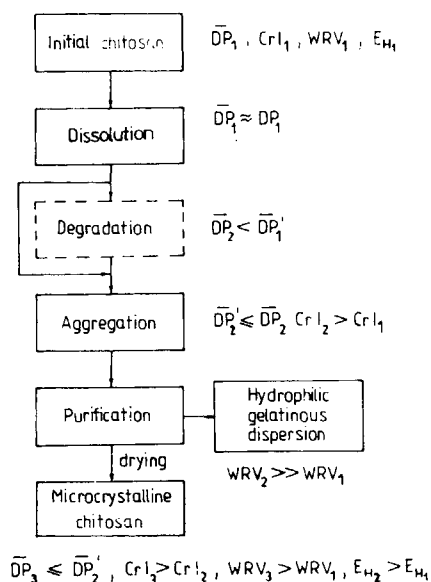
The present work was carried out with the aim of the preparation of microcrystalline chitosan by aggregation of glucosamine macromolecules from aqueous solutions of organic acids. Properties of the prepared product have been determined in conjunction with its supermolecular structure.

## EXPERIMENTAL

### Materials

The standard chitosan flakes originated from the Antarctic krill characterized by moisture of 7.5%, deacetylation degree of 64.0%, crystallinity index (CrI) of 58.9%, water retention value in solid state,  $\text{WRV}_s = 123.5\%$ , and

## MANUFACTURE OF MICROCRYSTALLINE CHITOSAN



Scheme 1

average molecular weight of  $5.05 \times 10^5$ , donated by the Sea Fishing Institute, Gdynia, Poland, were used in these studies. Other chemicals were of general purpose reagent grade and were used as supplied.

### Methods

**Preparation of Microcrystalline Chitosan in the Forms of Gel and Powder.** Microcrystalline chitosan (MCCh) was prepared according to Scheme 1<sup>7</sup>. To prepare the hydrogel, proper quantities of chitosan flakes were dissolved in aqueous solutions of acetic acid of concentrations 2% or 4% wt at a temperature of 20°C for 4 h, and the obtained polymer solutions were filtered to remove undissolved or gelatinous particles. Chitosan solutions of concentrations 0.5–2.0% by wt were used for investigations.

When the thermal degradation process was used the chitosan solution was contained in a glass flask provided with a thermometer and a stirrer and placed in a water bath. The degradation was carried out for 3 h at a temperature of  $70 \pm 0.1^\circ\text{C}$ . Then the flask content was immediately cooled.

The preparation of MCCh hydrogel via the aggregation of glucosamine macromolecules was accomplished by introducing an aqueous NaOH solution with a concentration from 2.95% to 29.5% by wt into the chitosan solution at  $20 \pm 0.1^\circ\text{C}$  while stirring at 150 rpm until pH 8.0 of the medium was obtained. Then the precipitated product was washed with a distilled water until the conductivity of a filtrate was the same as that of a water used.

The obtained hydrogel products were analyzed and dried under reduced pressure at a temperature of 60°C or in the air-spray drier if a microcrystalline powder was to be obtained.

**Analytical Methods.** The average molecular weight of MCCh and initial raw material was determined by the viscosimetric method in a solution containing 0.2M of acetic acid, 0.1M of sodium chloride, and 4.0M of urea, measuring the limiting viscosity number and using  $K = 8.93 \times 10^{-4}$  and  $\alpha = 0.71$  in the Mark-Houwink equation, as given by Lee.<sup>1,8</sup>

The degree of deacetylation of chitosan was measured by the IR spectrophotometric method<sup>9</sup> using a Specord 71 IR within a frequency range of 4000–800  $\text{cm}^{-1}$ . The deacetylation degree (DD) was found from the relationship

$$\text{DD} = 100 - \left[ \frac{(A_{1660}/A_{3450}) \times 100}{1.33} \right] (\%)$$

where  $A$  = absolute height of the absorption band of the amide group ( $A_{1660}$ ) and of hydroxyl group ( $A_{3450}$ ), respectively.

The water retention value (WRV)<sup>10</sup> of chitosan in hydrogel (WRV<sub>g</sub>) and solid (WRV<sub>s</sub>) forms was determined by submerging a tested sample in excess distilled water. After 20 h the water was separated, and the sample was filtered off with a filtration fabric and then centrifugated for 10 min at 4000 rpm. The sample weight was determined after centrifuging ( $m_1$ ) and then after drying to constant weight at 105°C ( $m_0$ ). The water retention value was found from the relationship

$$\text{WRV} = \frac{m_1 - m_0}{m_0} \times 100 (\%)$$

The crystallinity of an initial raw material and of the obtained solid products was determined by the X-ray diffraction method using a Drom-1 Diffractometer at a recording rate of 2°/min. The crystallinity index (CrI) was found by a method used for cellulose<sup>11</sup> according to the relationship

$$\text{CrI} = \frac{I_{002} - I_{\text{am}}}{I_{002}} \times 100 (\%)$$

where  $I_{002}$  = crystallinity diffraction pattern intensity at  $2\theta \approx 20^\circ$  and  $I_{\text{am}}$  = amorphous diffraction pattern intensity at  $2\theta \approx 12^\circ 30'$ .

The energy of hydrogen bonds,  $E_{\text{H}}$ , of chitosan samples was determined by IR spectrophotometry,<sup>12,13</sup> using a Specord 71 IR. The values of  $E_{\text{H}}$  were found from the obtained absorption spectra according to an equation:

$$E_{\text{H}} = \frac{1}{k} \frac{V_0 - V}{V_0} (\text{kcal})$$

where  $V_0$  = standard frequency corresponding to the vibration of free OH groups ( $3650 \text{ cm}^{-1}$ ),  $V$  = frequency corresponding to the vibration of bound OH groups, and  $k$  = constant equal to  $1.6 \times 10^{-2} \text{ kcal}^{-1}$ .

The appearance of gelatinous dispersion as well as a powder forms of product was studied by the optical microscopy and scanning electron mi-

croscopy (SEM). Microphotographs on the optical microscope of Carl Zeiss type of magnification of 100—as well as 620 times—were made. Adequately, an SEM microphotograph on the scanning electron microscope of Jeol 15 M 50 A type for magnification of 1000 times was obtained.

## RESULTS AND DISCUSSION

The process of preparation of microcrystalline chitosan by means of alkaline regenerating solution involves a number of phenomena, such as neutralization, coagulation, and aggregation of macromolecules. They are responsible for the specific properties of a product and mainly for its supermolecular structure different from that of the initial raw material.

It may be assumed that the properties of microcrystalline chitosan depend on several parameters of the preparation process, namely,

$$\text{properties of MCCh} = f(A, B, C, D, \dots)$$

where  $A$  = properties of the initial chitosan,  $B$  = parameters of the degradation process,  $C$  = parameters of the aggregation process, and  $D$  = parameters of the drying process.

The considered process is based on the aggregation of glucosamine macromolecules taking place due to specific neutralization of the chitosan salt, accompanied by coagulation and precipitation of hydrogel.

The preparation of MCCh is similar, in principle, to the preparation of microcrystalline cellulose from diluted viscose.<sup>3,14</sup> However, the formation of microcrystalline cellulose by coagulation of cellulose xanthate and regeneration of cellulose is only to some extent comparable with the phenomena taking place during the MCCh formation (Scheme 1).

The flow behavior of any polyelectrolyte polysaccharide is dictated by its overall molecular conformation and the degree of hydrogen bonding or electrostatic repulsion between neighboring chain segments.<sup>15-18</sup> Chitosan has a relatively more flexible backbone than other polysaccharides, which allows it to occupy a more compact "quasiglobular" conformation or a typical random coil conformation, depending on the solution conditions. An increase in chitosan concentration is expected to induce an increase in chain interpenetration affecting inter- and intramolecular interactions.<sup>15,16</sup> The neutralization of charges on the polyelectrolyte brought about salt ions causes Van der Waals forces to overcome the electrostatic monomer–monomer repulsive interactions. This phenomenon allows hydrogen bonding between neighboring segments to decrease the overall chain flexibility of the glucosamine macromolecules.<sup>15</sup> The present study has been aimed at the determination of the effect of some parameters of the MCCh preparation on properties of the product obtained.

Some properties of MCCh prepared by aggregation of glucosamine macromolecules, which are finally compressed due to drying, depend undoubtedly on the concentration of polymer solution. The results confirming this effect are given in Table I.

It follows from these results that increasing the chitosan concentration brings about a corresponding rise in the water retention value of the hydrogel form ( $WRV_g$ ) and a relatively small decrease in an average molecular weight

TABLE I  
Effect of Chitosan Solution Concentration on the Product Properties<sup>a</sup>

WRV (%) Chitosan concentration <sup>b</sup>			$\overline{MW}$ ( $\times 10^{-5}$ )	CrI (%)	$E_H$ (kcal)
	Gelatinous dispersion	Solid			
—	—	123.5	5.05	58.9	2.91; 3.93
0.5	786	349	4.89	70.6	3.85; 4.20; 4.53
1.0	741	309	4.76	84.2	3.85; 4.28; 4.60
1.5	892	346	4.71	82.2	3.94; 4.37; 4.62; 5.31
2.0	1054	337	4.58	75.0	3.68; 4.37; 4.79

<sup>a</sup>Using 5.9% aqueous sodium hydroxide solution.

<sup>b</sup>In 4% aqueous acetic acid solution.

of the product. WRV is a parameter describing the ability of product structure to retain water and reflecting, at the same time, an increase of the product internal surface. On the other hand, the WRV of dried product and the hydrogen bond energy show no significant relationships. The differences in the properties of obtained product and those of the initial raw material are worth noting here.

Based on these results (Table I), the concentration of chitosan in the solutions affects directly the interactions between the macromolecules of glucosamine responsible for corresponding changes in the crystallinity index value of product. At the same time, the parameter under consideration is associated with the technological conditions of MCCh preparation process such as the solution viscosity, shearing stress, etc.

The use of alkaline solution to neutralize a chitosan salt in association with coagulation and precipitation of glucosamine macromolecules from acid solutions provides suitable conditions to prepare a product with properties dependent on the concentration of sodium hydroxide solution.

Figure 1 shows the relationship between MCCh properties and concentration of the regenerating alkaline solution. It is worth noting the the increase

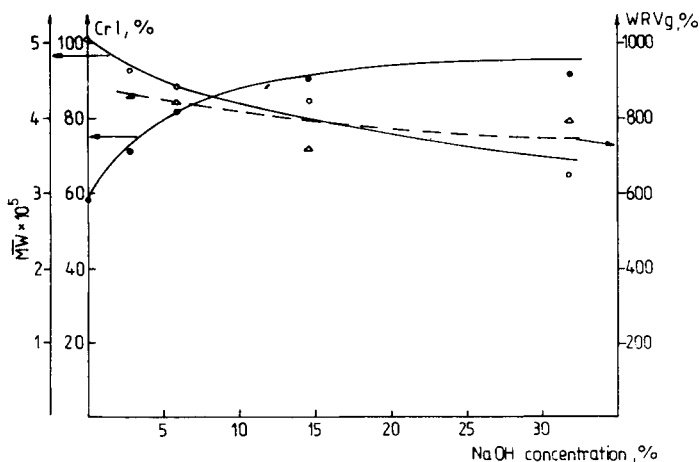


Fig. 1. Effect of sodium hydroxide solution concentration on the microcrystalline chitosan properties (1% chitosan solution in 2%  $\text{CH}_3\text{COOH}$ ).

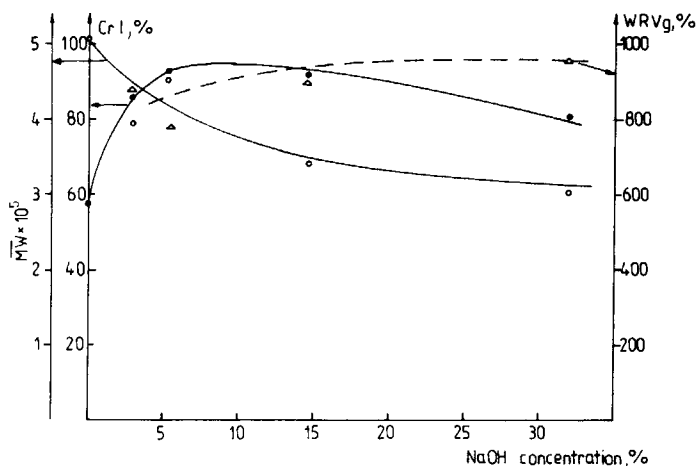


Fig. 2. Effect of sodium hydroxide solution concentration on the microcrystalline chitosan properties (1% chitosan solution in 2%  $\text{CH}_3\text{COOH}$ , degradation carried out in  $70^\circ\text{C}$  for 3 h).

in sodium hydroxide concentration considerably affected an increase of the crystallinity index and a decrease of the average molecular weight as well as the  $\text{WRV}_g$  of the product.

Similar results obtained in a case of the chitosan degradation performed with acetic acid solution at  $70^\circ\text{C}$  for 3 h are given in Figure 2. Except  $\text{WRV}_g$  the changes in the remaining properties are similar to those previously observed.

Table II contains the results showing an effect of sodium hydroxide concentration on hydrogen bonds energy and the  $\text{WRV}_g$  of the product obtained. These parameters are increased with a rise of the sodium hydroxide solution concentration.

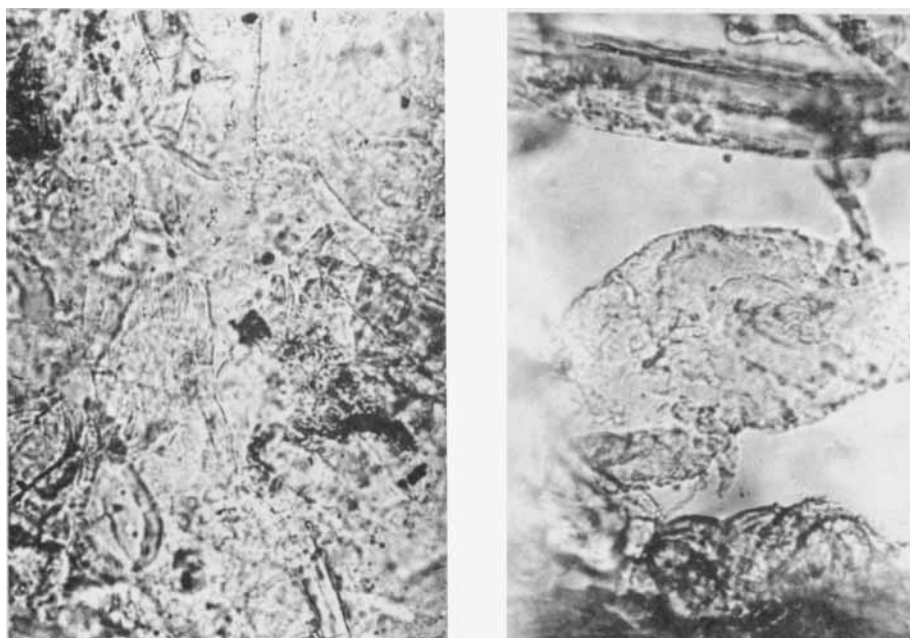
To sum up this stage of the study, one can state that the effect of an alkaline solution concentration on the MCCCh properties should be explained in terms of the rates of neutralization, coagulation, or precipitation as well as of degradation taking place at the same time. The latter may be a result of local cleavages of the macromolecules. A relatively low neutralization rate of the acetic acid solution containing chitosan salt leading to coagulation and

TABLE II  
Some Properties of MCCCh Obtained in Different Conditions<sup>a</sup>

Sodium hydroxide concentration (%)	Nondegraded		Degraded <sup>b</sup>	
	$\text{WRV}_g$ (-)	$E_H$ (kcal)	$\text{WRV}_g$ (%)	$E_H$ (%)
—	123.5	2.91; 3.93	—	—
2.95	276.5	3.42; 3.99	265.5	3.77; 4.02
5.90	286.5	3.85; 4.28	222.0	3.85; 4.45
14.75	282.0	3.68; 3.94	312.0	3.89; 4.37
29.50	517.5	3.93; 4.20	246.0	4.11; 4.28

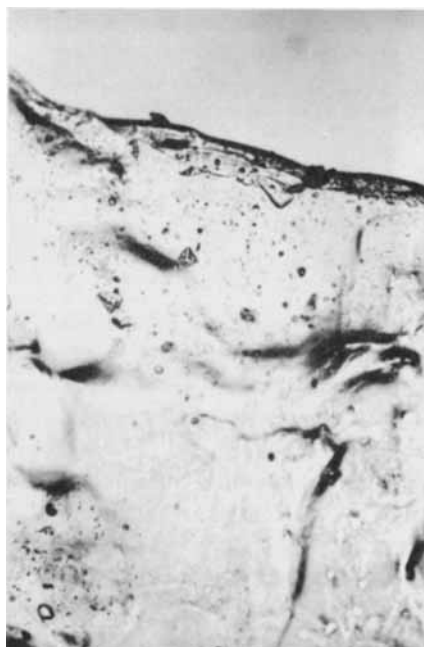
<sup>a</sup>1% chitosan solution in 2% aqueous acetic acid.

<sup>b</sup>Degradation was carried out for 3 h at  $70^\circ\text{C}$ .



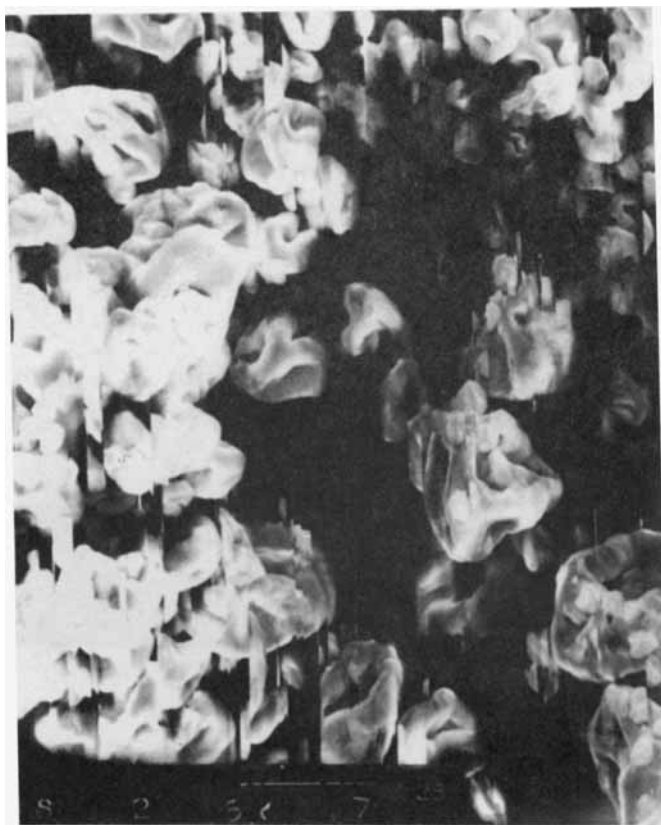
(a)

(b)



(c)

Fig. 3. Optical microscope microphotographs of water-swollen microcrystalline chitosan aggregates ( $a, b \times 100, c \times 620$ ) as well as SEM microphotograph ( $d \times 1000$ ) of air-spray-dried microcrystalline chitosan powder.



(d)

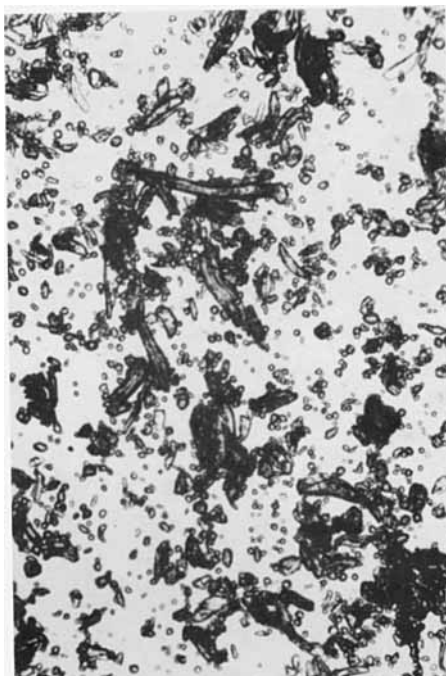
Fig. 3. *Continued from the previous page*

aggregation of the macromolecules allows above processes to proceed mildly and consequently to form a product with high energy of hydrogen bonds and supermolecular structure of high crystallinity index (Figs. 1 and 2; Tables I and II).

In a case of intentional degradation of chitosan during preparation of MCCh, the observed differences, as compared with standard process (Figs. 1 and 2; Table II), are associated with supermolecular structures of the product. It should be noticed that an increase in the crystallinity index of product is accompanied by decrease in  $WRV_g$  (Fig. 2). This phenomenon may result from the aggregation of macromolecules with lower average molecular weight and higher energy of hydrogen bonds between them than those of the undegraded chitosan, and of higher ability of the structure for compression (Table II). A similar phenomenon was observed previously in a case of producing some modifications of microcrystalline cellulose.<sup>1,2,14</sup> Such extended investigations are in progress and will be published soon.

To illustrate the properties of product, it was examined by means of optical microscopy as well as of scanning electron microscopy (Fig. 3). The microscopic pictures show characteristic aggregates in the aqueous dispersion of hydrogel [Figs. 3(a)–(c)]. Figure 3(d) shows an SEM microphotograph of powdered MCCh prepared by air-spray drying. For purposes of comparison,





(a)

Fig. 4. Microphotographs of the Somichel F type microcrystalline cellulose of: (a) water-swollen microcrystalline cellulose aggregates (optical microscope  $\times 100$ ); (b) air-spray dried powder (SEM  $\times 1000$ ).

Figure 4 illustrates microcrystalline cellulose of the Somichel F type in the forms of dispersion and powder.

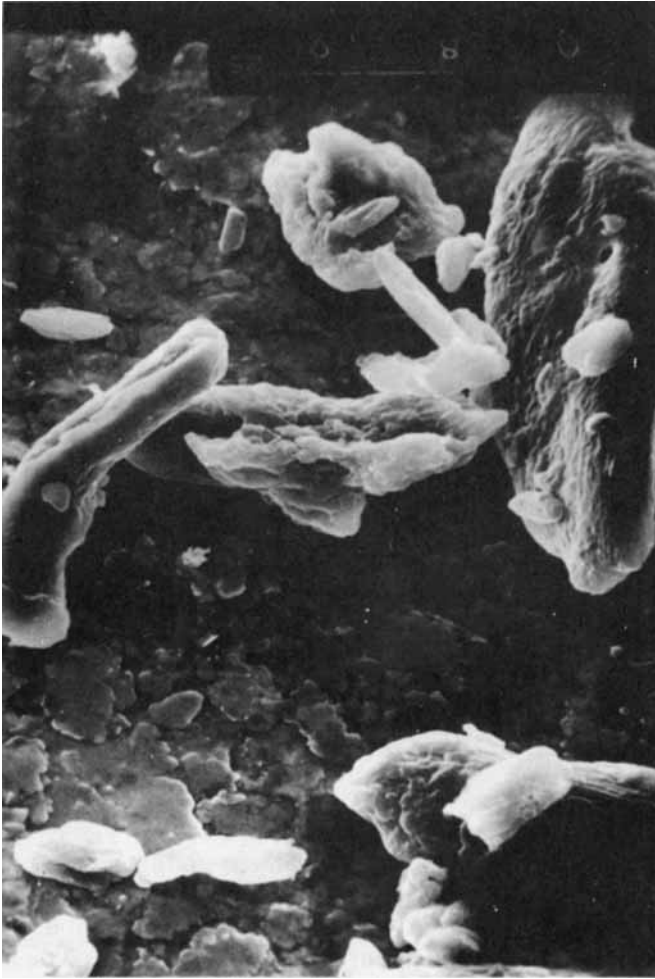
The results given in Table III point to high comparable purity of both products. Microcrystalline chitosan of Chicol type produced on an experimental scale was characterized by higher values of the water content, WRV, CrI, and the average molecular weight as compared with the Polish microcrystalline cellulose.

Microcrystalline chitosan in a form of powder prepared from the hydrogel dispersion by air-spray drying (Table III; Figs. 3 and 4) showed different shape of particles as compared with the Somichel F microcrystalline cellulose. This is due to differences in properties of the natural polymers under investigation such as film-forming behavior, elasticity, or shape of hydrogel particles as well as to differences in the preparation methods.

Microcrystalline chitosan of the Chicol type was characterized by oval shape of particles [Fig. 3(d)] with an average size within a range of 5–20  $\mu\text{m}$  while the Somichel F microcrystalline cellulose showed elongated shape of particles with an average size from 10 to 100  $\mu\text{m}$  (Fig. 4).

IR absorption spectra of MCCCh and of initial raw material are shown in Figure 5. One can observe an increase in the optical density<sup>19</sup> of absorption band at about 1380  $\text{cm}^{-1}$  as compared with initial chitosan. This can be explained by a corresponding rise of the product crystallinity.<sup>20</sup>

The preparation process of MCCCh based on a use of alkaline solution did not affect significantly the deacetylation degree of produce due to the short



(b)

Fig. 4. *Continued from the previous page*

time of its action at a relatively low temperature. At the same time the yield of MCCh was from 80 to 95% for a hydrogel and from 70 to 90% for a powder product obtained by the air-spray drying.

Owing to a use of the alkaline solution, there are conditions suitable for approaching macromolecules or unstable aggregates, formation of the second order bonds which accompany an aggregation, and, in a further stage formation of stable structures of higher degree of ordering (Tables I and II; Figs. 1-3). Owing to the above processes and to the chitosan ability to form of hydrogen bonds,<sup>2,6,15</sup> it is possible for the glucosamine macromolecules to come nearer to forming stable bonds.

The preparation of microcrystalline chitosan from acidic solutions of this polymer by mild neutralization and regeneration, including the removal of low molecular ionic byproducts, makes it possible to obtain a product of high crystallinity. This process enables maximum mutual approach of the macro-

TABLE III  
Powdered Microcrystalline Chitosan<sup>a</sup> and Cellulose<sup>b</sup> Properties

Type of microcrystalline polymer	Form and color	Water content (%)	pH	$\overline{MW}$	WRV <sub>s</sub> (%)	WRV <sub>g</sub> (%)	CrI (%)	Average particles dimension ( $\mu\text{m}$ )	Heavy metal content (ppm)
Microcrystalline chitosan of Chicol	Powder, light grey	13.4	7.0	$3.64 \times 10^5$	321	855	94.0	5-20	$\leq 10$
Microcrystalline cellulose of Somical F	Powder, white	5.5	6.5	$1.05 \times 10^4$	70	120	76.0	10-100	$\leq 10$

<sup>a</sup> Produced in development scale in 4% CH<sub>3</sub>COOH using 4% NaOH, without degradation.

<sup>b</sup> Pharmaceutical type of Polish MCC.

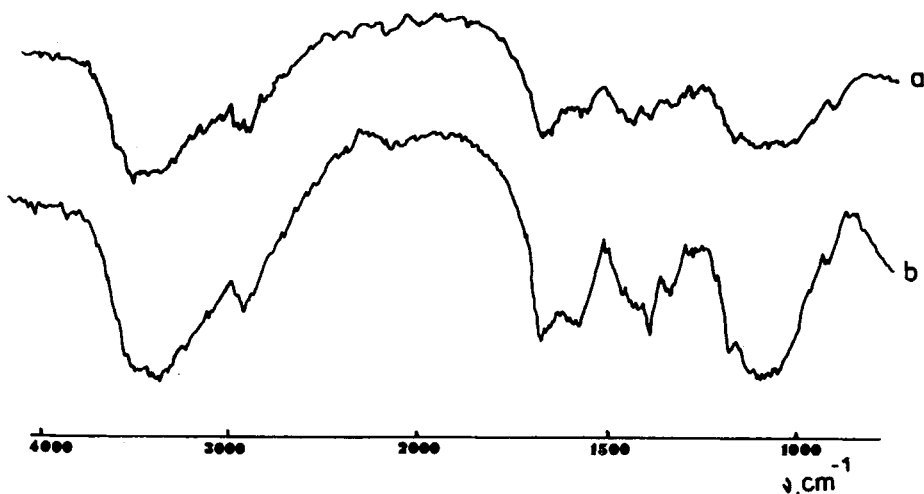


Fig. 5. Infrared spectra of (a) initial chitosan as well as (b) microcrystalline chitosan obtained from 1% chitosan solution in 2%  $\text{CH}_3\text{COOH}$  using 2.95% aqueous sodium hydroxide solution.

molecules in an aqueous medium, while further compression of the product structure can take place in drying, particularly by means of the air-spray drying as in a case with microcrystalline cellulose (Table III).

Microcrystalline chitosan, owing to its specific properties, can be used among other things in medicine, pharmacy, cosmetics, or agriculture.<sup>21-23</sup> The various possible applications of MCCh in a form of hydrogel or powder bring about a progress in studies on its properties, structure, and modifications.

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