

# Investigation of Properties of Chitosan Obtained by Solid-Phase and Suspension Methods

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**ABSTRACT:** The properties and fractional composition of chitosan prepared by the suspension method and by solid-phase deacetylation of chitin under conditions of joint action of high pressure and shear deformations was studied. Elemental analysis, particle size distribution analysis, viscosimetry, potentiometry, and other experimental techniques were used in the study. The specific features of chitosan obtained by solid-phase extrusion were examined. © 1998 John Wiley & Sons, Inc. *J Appl Polym Sci* 70: 927–933, 1998

**Key words:** chitosan; solid-phase method; suspension method

## INTRODUCTION

Chitosan is a product of deacetylation of chitin, which is a poly-*N*-acetylglucosamine and is the second after cellulose natural carbohydrate polymer in abundance. Chitosan is usually prepared by treating chitin by concentrated aqueous NaOH solutions at elevated temperatures. At that, the deacetylation and part depolymerization of chitin with the formation of chitosan takes place (see Scheme 1).

Working out new procedures for the synthesis of chitin derivatives is one of the promising trends in modern chemistry of the polysaccharides. One of them is the production of chitosan under conditions of joint action of high pressure and shear deformations. This method can be realized in a solid phase that increases the ecological safety of the process and decreases the consumption of reagents and amount of sewage water.

It is known that the reactivity of polysaccharides largely depends on the specific features of

their supermolecular structure. The changes that take place in the cellulose structure subjected to the joint action of high pressure and shear deformation and chemical modification of cellulose under these conditions were examined in ref. 1.

The aim of this study was a systematic examination of the properties of chitosan obtained from chitin under conditions of shear deformations.

## EXPERIMENTAL

### Suspension Production of Chitosan

The suspension reaction of chitosan production was carried out in a flask purged with argon by stirring a suspension of chitin in a 50% NaOH solution according to previous works.<sup>2</sup>

### Sample Treatment under Pressure

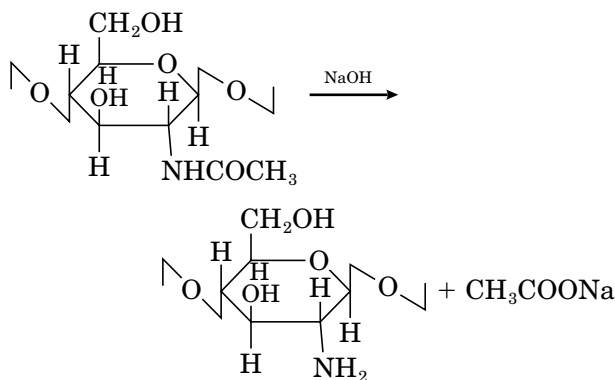
The reaction of chitosan production from chitin was conducted in a two-screw extruder ( $D = 40$  mm) with controlled heating. A mixture of chitin and solid sodium alkali taken in different ratios

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Scheme 1

was fed to the extruder. When the reaction mass passes through extruder, it is subjected to the joint action of high pressure and shear deformation at different temperatures. The obtained reaction mass is a mixture of chitosan of various degrees of deacetylation, depending on process conditions. The mixture was washed by water to neutral reaction and dried under room temperature.

#### Determination of Degree of Deacetylation (DD)

The degree of deacetylation of chitosan was calculated as the ratio of contents of the amino nitrogen  $[N]_{\text{am}}$  and the total nitrogen  $[N]_{\text{tot}}$ . The  $[N]_{\text{am}}$  value was determined by potentiometric titration of a polymer solution in hydrochloric acid with NaOH on an RTS-822 automated titrator (Radiometer, Denmark). Figure 1 shows a typical titration curve, on which the pH levels corresponding to the beginning ( $\alpha = 1$ ) and to the end ( $\alpha = 0$ ) of titration of the  $\text{NH}_4^+\text{Cl}^-$  groups of chitosan are indicated, where

$$\alpha = \frac{[\text{NH}_3^+]}{[\text{NH}_3^+] + [\text{NH}_2]}$$

is the degree of amino group ionization.

The  $[N]_{\text{am}}$  value was calculated by the formula

$$[N]_{\text{am}} = \frac{V \cdot [\text{NaOH}] \cdot 14}{q} \cdot 100\%$$

where  $V$  is the volume of NaOH solution consumed for the titration of  $\text{NH}_4^+\text{Cl}^-$  groups (mL) and  $q$  is the chitosan weight (g). The total nitrogen content  $[N]_{\text{tot}}$  was determined on an EA-1108 elemental analyzer (Carlo Erba, Italy).

#### Determination of Molecular Weight of Chitosan

Intrinsic viscosity of the chitosan solutions was measured in a mixed (0.2 M  $\text{CH}_3\text{COONa}$  + 0.3 M  $\text{CH}_3\text{COOH}$ ) solvent at 25°C with an Ubbelohde viscometer; the initial concentration of chitosan solution was 0.2–0.5 g/dL. The molecular mass of chitosan was calculated according to work.<sup>3</sup>

$$[\eta] = 1.38 \times 10^{-4} M^{0.85},$$

where  $[\eta] = \lim_{c \rightarrow 0} (\eta_{\text{sp}}/c)$  and  $\eta_{\text{sp}} = (\eta - \eta_0)/\eta_0$ ,  $\eta$ , and  $\eta_0$  is the viscosity of the solution and the solvent, respectively.

#### Distribution of Chitosan Particles in Size and Shape

The samples of chitosan were dried to the constant weight at 105°C and then were spread on the glass plate to obtain microphotographs of particles using research transmission optical microscope "Jenaval," Carl Zeiss, Jena at  $\times 65$  magnification.

The distribution of chitosan particles in size and shape was examined with the aid of GALLERY software for the analysis of optical images. The reduced size of an object was calculated as the diameter of a circle whose area was equal to that of the object.

The shape factor of a particle  $n$  was determined from the ratio of the area  $S$  to the squared parameter  $P$  according to previous works:<sup>4</sup>

$$n = 4\pi S/P^2$$

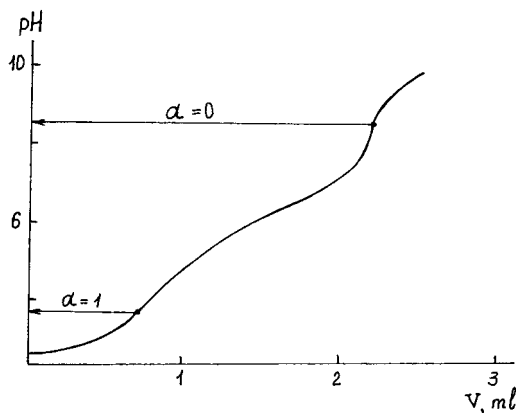


Figure 1. Titration curve of a 0.05% solution of sample 2 (5.5 mL) in HCl.

**Table I** Conditions of Synthesis and Characteristics of Chitosan Samples

Sample No.	Method of Synthesis	Molar Ratio Chitin : NaOH : H <sub>2</sub> O	T, °C	Reaction Duration, h	Solubility, % <sup>a</sup>	DD		[η], dl/g
						Soluble Fraction	Insoluble Fraction	
1	Suspension	1 : 20 : 43	150	2.0	98	0.82	0.18	7.0
2	Solid state <sup>b</sup>	1 : 5.0 : 11	180	0.1	96	0.81	0.19	1.2
3	Solid state	1 : 4.3 : 13	150	0.1	86	0.78	0.22	1.2

<sup>a</sup> Solubility in 2% acetic acid.

<sup>b</sup> The solid state of the system is virtually not changed by water present in the reaction mixture (10–13 mol H<sub>2</sub>O per 1 mol chitin, or ~ 1 g/g).

The particle shape factor is equal to 1 for ideal round objects and is reduced with increasing object asymmetry.

#### Fractionation of Chitosan by Method of Fractional Precipitation<sup>5</sup>

The solutions for fractionation were prepared by acidification of suspension aliquots (15 g) with 1 M HCl solution until obtaining pH 3.6, after which the volume was increased to 100 mL by adding water with continuous stirring. The insoluble polymer fraction was separated by centrifugation and dried to a constant weight. The fractional precipitation was realized by adding of portions of isopropanol. The precipitated fraction was separated by centrifugation, washed with a water–isopropanol mixture, and dried.

#### Fractionation of the Chitosan by Method of pH Variation

For the chitosan fractionation by method of pH variation, the solution was gradually neutralized by adding NaOH (0.5 mol/L), and the precipitated fractions were separated as described above.

## RESULTS AND DISCUSSION

Table I lists the characteristics of the three chitosan samples. Sample 1 was obtained by conventional suspension technique,<sup>2</sup> while samples 2 and 3 were obtained by the extrusion method based on the solid-phase deacetylation of chitin.

As is seen from these data, sample 2 (obtained by the solid-phase synthesis) is virtually identical to sample 1 (synthesized by the conventional suspension deacetylation method) with respect to im-

portant characteristics such as the solubility and degree of deacetylation.

The results of chitin deacetylation under shear deformation at different initial ratios chitin : NaOH and different temperatures are presented in Table II. As shown from the table, the increase of NaOH excess leads to a growing degree of deacetylation and the solubility of obtained products.

The five times NaOH mol excess is enough to produce a high deacetylated product with a solubility of about 90%, although known suspension procedures use an excess mol of no less than 10. So the alkali consumption is reduced in the proposed method up to 75–80%.

The most important factor that influenced the chitosan yield is temperature. The degree of deacetylation increased from 0.39 to 0.98 as the temperature increased from 25–200°C at a mol ratio of reagents 1 : 5.

The temperature range 180–200°C and five times excess of NaOH seems to be optimal conditions to obtain high deacetylated and high soluble chitosan on the used equipment. The great excess of NaOH obviously leads to blocking of reaction centers of chitin, and that decreases the product yield; at high temperatures, the combustion of reaction mixture is possible.

The MW of chitosan samples obtained was approximately 40,000–60,000. Generally, the decrease of MW highly depends on temperature, being more intensive at a temperature increase. The efficient decrease of degree of polymerization of polysaccharide derivatives and other polymers obtained under conditions of a joint action of high pressure and shear deformations is a general feature caused by specificity of this kind of action. A decrease in molecular weight (and accordingly in viscosity) is associated with extensive degradation of the initial chitin. A decrease in solubility is

**Table II Parameters of Chitosan Obtained by Extrusion Method**

Conditions of Process		Parameters of Chitosan Samples		
Molar Ratio of Chitin : NaOH	Temperature, °C	DD	Solubility, %	MW, 10 <sup>-4</sup>
1 : 3	120	0.09	5.0	6.05
1 : 3	180	0.63	62.0	4.70
1 : 3 <sup>a</sup>	180	0.73	70.0	4.50
1 : 3	200	0.70	65.0	4.52
1 : 5	25	0.33	36.0	6.72
1 : 5	100	0.40	39.0	4.65
1 : 5	160	0.59	60.0	7.22
1 : 5	180	0.81	78	7.75
1 : 5 <sup>a</sup>	180	0.90	87	6.00
1 : 5	200	0.98	90.0	4.30
1 : 10	25	0.12	5.0	4.06
1 : 10	50	0.27	8.5	4.23
1 : 10	100	0.43	46.0	6.00
1 : 10	160	0.76	77.0	7.00

<sup>a</sup> Double treatment of reaction mixture in extruder.

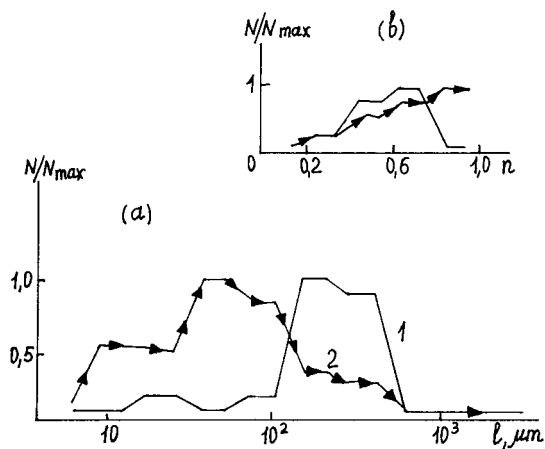
related to the difficulties in providing uniform distribution of the reagents within the short time taken by the reaction (minutes), which leads to molecular heterogeneity of the products.

The different character of change in MW of chitosan in presence of NaOH at various initial ratios chitin : NaOH with temperature (decrease at ratio 1 : 3, nonmonotonic character at ratio 1 : 5, and increase at ratio 1 : 10) may be explained by various numbers of reaction centers of chitosan under these conditions. At the minimum excess of NaOH (1 : 3 ratio) the destruction is increased with temperature and the MW is decreased. At the maximum excess of NaOH (1 : 10 ratio) the reaction centers are blocked with excess NaOH, and with a temperature destruction is decreased (MW is increased). The 1 : 5 ratio is intermediate, and is characterized by a non-monotonic MW change.

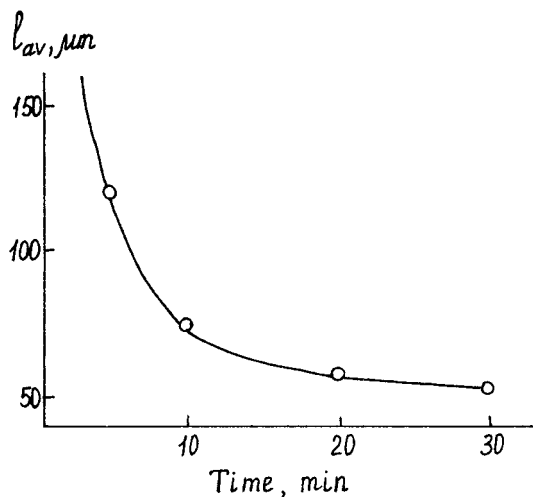
It is quite natural that the degrees of dispersibility of chitosan samples obtained by suspension and solid-phase methods are significantly different. As is seen from Figure 2, sample 1 shows large (130–500  $\mu\text{m}$ ) and asymmetric particles, whereas in the extruded sample 3 the particles are smaller (10–130  $\mu\text{m}$ ), more uniform in size, and round shaped.

The effect of extrusion grinding conditions (duration and temperature) on the properties of the chitosan and on the size of chitosan particles were specially studied. The most dramatic decreases in

the size of the chitosan particles take place during the first 5–10 min of the process (Fig. 3). Chitosan ground in the extruder for this period of time is similar in particle size distribution to chitosan obtained by the solid-phase method in the extruder. Increasing the duration of grinding further (increasing the number of extrusion runs) has almost no effect on the particle size, but predominantly increases their uniformity and decreases their asymmetry (oval particles become round). It is noteworthy that the grinding efficiency is the highest at room temperature (Fig. 4)



**Figure 2.** (a) Particle size distribution and (b) form factor distribution for (1) sample 1 and (2) sample 3.



**Figure 3.** Mean reduced particle size for sample 1 as a function of grinding duration at 180°C.

because at elevated temperatures, the polymer becomes less brittle.

At high temperatures a decrease in the molecular mass resulting from the grinding is slower. It is noteworthy that the variation of molecular masses with time is nonmonotonic. For example, after a single run at 25°C, the molecular mass decreases by another 10%, whereas when the number of runs is increased further and the zone of back mixing is formed, the molecular mass slightly increases. With an increase in the duration of extrusion grinding, the solubility of polymer decreases; the decrease is most pronounced (50% loss of solubility) when the grinding is conducted at 180°C.

The obtained data suggests that the destruction of the chitosan structure during extrusion grinding is obviously accompanied by intermolecular crosslinking of the polymer; at 180°C the crosslinking starts earlier.

Important characteristic of chitosan obtained by suspensional and solid-phase methods is the fractional composition, which is related to its physical and chemical inhomogeneity and determines the toxicity and, probably, the biological activity of chitosan-based polymeric drugs. This characteristic is especially important for the chitosan obtained by solid-phase synthesis of the reaction products.

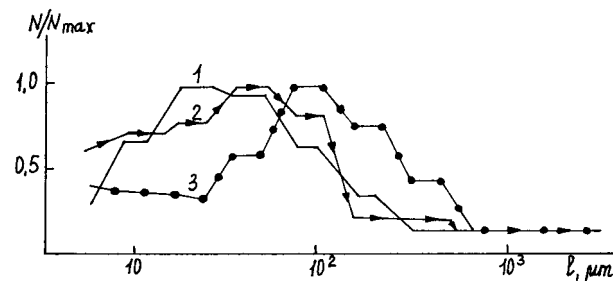
The partition of chitosan was performed by the method of fractional precipitation. It was believed that adding of organic solvent led to the precipitation of fractions differing mostly by the molecular weight, whereas the precipitation of the poly-

mer by pH variation separated the fractions with different degrees of deacetylation. The latter assumption was based on the notion that a fraction having a lower DD values and, hence, a smaller degree density on macroions, must exhibit a lower solubility at a given pH.

In Tables III and IV are given the characteristics of fractions obtained by portioning samples 1–3, and Figure 6 shows the molecular mass distributions for these samples.

As is seen from Table III, the difference between separated fractions of samples 1 and 2 is rather small, and comparable with the uncertainty of the determination of this characteristic. Therefore, we may conclude that the partitioning procedure yielded fractions differing by the molecular weight, and use these data for evaluating the molecular weight distribution of samples 1 and 2. The main difference between the two samples was that sample 1 (obtained by the suspension method) has a bimodal molecular mass distribution, while sample 2 (synthesized by solid-phase deacetylation) has a unimodal distribution (Fig. 6). Apparently the mechanical destruction of polymers under conditions of the joint action of pressure and shear deformations has a less selective character, leading to a low molecular-weight product with a comparatively narrow weight distribution.

Table IV gives the characteristics of fractions obtained with sample 3 partitioned by the method of pH variation in the chitosan solution. As is seen, the chitosan fractions separated as a result of pH variation exhibit no significant difference with respect to molecular weight, but show a clearly pronounced tendency of the degree of deacetylation to increase in the fractions precipitated at high pH values. At the same time, the DD values of the precipitated fractions differ by no more than 20%. Thus, despite the solid state of the initial components, the reaction of chitin



**Figure 4.** Particle size distribution for sample 1 ground at (1) 25, (2) 100, and (3) 180°C.

**Table III Characteristics of Various Fractions of Samples 1 and 2 Obtained upon Fractionation in the Water-HCl-Isopropyl Alcohol System**

$V_{\text{prec}}/V_{\text{S}}^*$	Fractional Yield, %	DD	$[\eta]$ , dL/g	$M \times 10^{-3}$
Sample 1				
0	—	0.82	7.0	343
0.5	20	0.82	8.0	402
0.8	55	0.81	7.5	372
1.0	3.0	0.82	6.6	320
1.5	2.0	0.81	6.2	297.5
2.0	5.0	0.83	5.5	258
2.5	8.0	0.83	5.2	242
3.0	4.0	0.82	4.6	209
Sample 2				
0	—	0.81	1.2	43.1
0.8	4.0	0.80	1.8	69.4
1.0	8.0	0.79	1.6	60.5
1.4	12.0	0.82	1.4	51.7
1.6	20.0	0.80	1.2	43.1
2.0	35.0	0.83	1.1	39.0
3.0	10.0	0.82	0.9	30.7

\* Volume ratio of precipitant and solution.

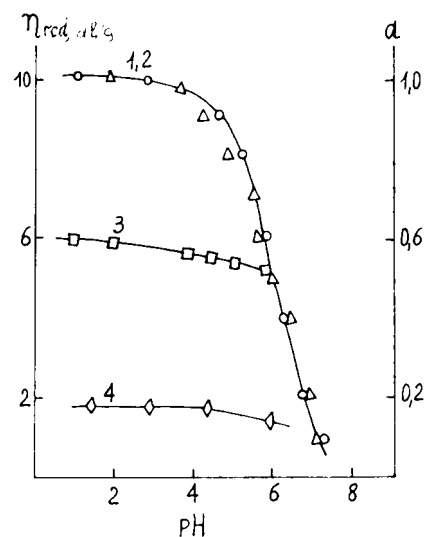
deacetylation under the plastic flow conditions leads to the products that are relatively homogeneous with respect to their chemical properties.

As is known from the literature<sup>6</sup> the viscosity of chitosan solutions slightly depends on pH media. To verify this effect the values of  $\eta_{\text{red}}$  of diluted HCl solutions of chitosan at various pH for samples obtained by the suspension (1) and by solid-phase methods (2) were determined. In Figure 5 the results of a comparative study of the degree of ionization of amino groups and the reduced viscosity  $\eta_{\text{red}}$  of hydrochloric solutions as

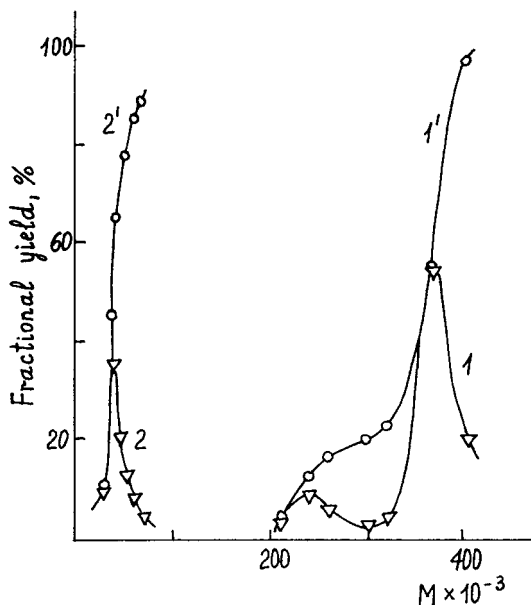
functions of pH samples 1 and 2 are presented. The  $\alpha$  vs. pH plots for the samples are absolutely identical (curves 1 and 2). The behavior of viscosity (curves 3 and 4) shows that the polymer pre-

**Table IV Characteristics of Various Fractions of Sample 3 Obtained by Variation of pH of the Chitosan Acetate Solution**

pH	Fractional Yield, %	DD	$[\eta]$ , dL/g	MW $\times 10^{-4}$
6.3	16	0.69	1.3	4.7
6.7	28	0.75	1.25	4.5
7.0	38	0.82	1.2	4.3
7.5	15	0.80	1.21	4.3
8.5	3	0.88	1.3	4.7
	100	0.78	1.20	4.3

**Figure 5.** The plots of (1,2) ionization degree and (3,4) viscosity vs. pH of 0.05% solutions of samples 1 (1,3) and 2 (2,4) in 0.1 M HCl.





**Figure 6.** Differential (1,2) and integral (1', 2') curves of molecular weight distribution of samples 1 (1,1') and 2 (2,2').

precipitates at the same pH value, for example, pH 6.1 + 0.2 for a chitosan concentration of 0.05% and anionic strength about 0.1 mol/L, which cor-

responds to  $\alpha = 0,5$ . Although the viscosity of the solutions of sample 2 is much lower compared to that of sample 1, the  $\eta_{sp}$  vs. pH curves have generally the same character. In the range of pH 1–6 (where  $\eta$  varies from 1 to 0.5), the drop of viscosity of both solutions did not exceed 10%, which is probably caused by a relatively high rigidity of the macromolecules of this polyelectrolyte.

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