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Chitin and Its Chemical Transformations

E.A.Plisko, L.A.Nud'ga, and S.N.Danilov

The present state of research in the field of the natural polysaccharide chitin and chitosan is described. The bibliography includes 225 references.

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I. INTRODUCTION

Despite the fact that chitin has been known for a long time[1,2] and occurs widely in nature, it has still been little investigated. There has been an increase of interest in chitin owing to the general development of the chemistry of polysaccharides and synthetic polymers. A number of studies have been published recently on chitin derivatives which are of practical value. There has been a considerable expansion of research also on the properties of deacetylated chitin (chitosan). A survey of the literature data is therefore of definite value, particularly since the available reviews[3-7] do not reflect fully the advances achieved in this field.

II. THE STRUCTURE AND PROPERTIES OF CHITIN

Chitin occurs in nature in the organisms of certain lower animals and plants, predominantly in crustacea[8-11], insects[12], and fungi[11-15]. It is always present in combination with other substances[16], such as proteins, inorganic salts, or polysaccharides. During its isolation in a pure form, the chitin-containing material is therefore treated with appropriate reagents[13,17-19], which decompose the secondary substances but do not affect the chitin macromolecule. Plant and animal chitins are identical, which has been confirmed by their chemical composition[20], specific gravity[21], specific optical rotation[22], and the repeat period obtained by X-ray diffraction[23].

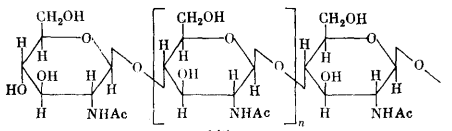
Chitin is one of the commonest polysaccharides in nature; marine crustacea alone yield thousands of millions of tons of chitin annually[24]. However, the industrial utilisation of chitin is difficult owing to its even distribution in nature.

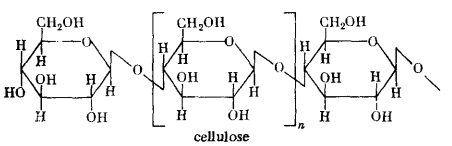
1. The Hydrolysis of Chitin

The complete acid hydrolysis of chitin leads to the formation of glucosamine and an equimolar amount of acetic acid[25]. After the hydrolysis of chitin with 70–72% sulphuric acid at room temperature for 2–3 days, monoacetylglucosamine was isolated[26]. This important discovery made it possible to determine the position of the acetyl group in monoacetylglucosamine. By hydrolysing chitin with highly concentrated hydrochloric acid, Zechmeister and coworkers[27,28] obtained not only the final chitin hydrolysis product (acetylglucosamine) but also a number of intermediate fractions, the acetylation of which gave a biose octa-acetate and chitotriose acetate[29]. They noted that the biose octa-acetate is identical with the chitobiose octa-acetate isolated in other investigations[30,31] by the acetolysis of chitin. Partial hydrolysis of chitin by concentrated hydrochloric acid in later studies[32-36] gave *N*-acetylchito-oligosaccharides. The chito-oligosaccharides were separated by thin-layer[37-39] and ion-exchange chromatography[40] as well as other methods[41-43]. Acetolysis of plant and animal chitins yielded chitobiose octa-acetate and chitotriose undeca-acetate, which are identical regardless of whether they are obtained from plant or animal chitin[35,36,44,45].

Chitin is very resistant to the action of bacteria[46], but micro-organisms decomposing it do exist[47-49]. It has been shown[50,51] that chitin is hydrolysed by the enzyme chitinase with formation of up to 80% of acetylglucosamine. A similar yield of acetylglucosamine has been obtained on cleavage of plant chitin with chitinase[52]. The above data suggests that chitin is a homogeneous linear polymer.

Comparison of the activation energies for the hydrolysis of chitin by nitric acid[53] and of cellulose by sulphuric acid[54] and of their repeat periods obtained by X-ray diffraction[23,55-57] as well as the formation of chitobiose octa-acetate by the acetolysis of chitin and the specificity of the action of chitinase show that the *N*-acetylglucosamine residues are joined via oxygen with the β-glucosidic 1,4-linkage and are alternately rotated by 180°, i.e. the chitin macromolecule has a structure similar to that of cellulose, but differs from it by the fact that there is an acetamide group at $C_{(2)}$ instead of a hydroxyl:





The oxidation of chitin by periodate also confirmed the above structure[58].

2. The Supermolecular Structure of Chitin

Many studies on the structure of chitin [59-71] led to the conclusion that chitin has a highly ordered stereoregular structure. Its X-ray diffraction pattern reveals a well-defined orthorhombic lattice [66]. The macromolecular chains of chitin are involved in strong hydrogen bonds both via the NH and CO group and via hydroxy-groups [68, 69, 72]. The crystal structure proposed by Carlström [68] has been confirmed by data obtained in a study [72] of the infrared spectrum of chitin. Detailed analysis of this spectrum confirmed the presence of the intermolecular C=O...N–H hydrogen bond along the fibre axis and the absence from the chitin crystals of free OH, NH, and C=O groups not involved in the hydrogen bond. The authors believe that the OH group at $C_{(6)}$, which exhibits free rotation, may be linked by an intramolecular hydrogen bond to the bridge oxygen and the nitrogen atom in a neighbouring glucosamine unit.

Recent X-ray diffraction studies [4, 69, 73] indicate the existence of chitin in three crystalline forms (α, β, and γ), differing in the size of the crystal lattice. It has been shown [74] that α-chitin is orthorhombic and its unit cell contains fragments of two chains only, arranged in opposite directions. All the forms are stable in boiling 5% KOH or NaOH solution [75], but their treatment with 6 *N* HCl leads to the conversion of the β- and γ-forms into the α-form [75]. Similar conversions have been observed also by other workers [11, 73].

3. The Properties of Chitin

Despite the similarities of the structures of chitin and cellulose, they differ significantly. In contrast to cellulose, chitin swells only slightly in solutions of alkalis and does not dissolve in the solvents used for cellulose (solutions of the amminecopper complex, the iron–tartrate–sodium complex, benzyltriethylammonium hydroxide, and the cadmium–ethylenediamine complex), probably due to the absence from chitin of two adjacent hydroxyls, which in the case of cellulose are responsible for the formation of molecular compounds [76, 77].

At the same time, chitin dissolves comparatively readily in concentrated hydrochloric and sulphuric acids [78, 79], from which it can be isolated almost unaltered [80]. Nitric and phosphoric acids also dissolve chitin [81, 82]. When chitin is dissolved in acids, gradual hydrolysis takes place, as a result of which a glucosamine salt is formed. Chitin solutions are laevorotatory; in hydrochloric acid with a specific gravity of 1.16, the initial specific rotation is $-14.7°$ and the final rotation is $+56°$ (which corresponds to the rotation of the glucosamine hydrochloride formed [22]). In view of this, chitin solutions in acids are unstable, their viscosity varying with time, so that it is impossible to determine the true molecular weight by viscometric, osmometric, light scattering, or sedimentation methods. An attempt has been made to estimate the molecular weight of chitin by measuring its viscosity in 50% nitric acid [81] and by extrapolating the viscosity to zero dissolution time. By comparing the values obtained with the analogous viscosities of wood cellulose in amminecopper solution, the authors [81] concluded that chitin and wood cellulose have similar molecular weights; this method is only approximate.

The method of copper numbers (determination of the content of terminal aldehyde groups) was used by Iterson [83] to estimate the degree of polymerisation (DP) of chitin. According to his data, the degree of polymerisation of chitin corresponds to 103 units. However, in another study [84] this method gave DP = 66. The method is comparatively rapid and can be used to characterise the variation of the degree of polymerisation of chitin. However, it does not yield unambiguous results owing to the polydispersity of the specimen and the possible oxidation of the terminal aldehyde groups to carboxy-groups. According to the data of other investigators [85-87], the degree of polymerisation of chitin varies from several hundreds to thousands of *N*-acetylglucosamine residues.

Chitin dissolves on heating in concentrated solutions of certain salts [87, 88] and the latter can be arranged in the following sequence in terms of their dispersing capacity [88]:

$$LiSCN > Ca(SCN)_2 > CaI_2 > CaBr_2 > CaCl_2.$$

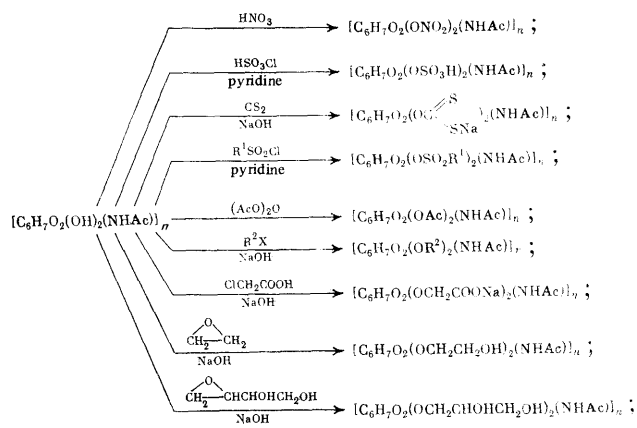
Partial dissolution of chitin has been observed in mixtures of dimethylformamide and nitrogen dioxide [89].

III. REACTIONS IN THE CHAINS OF THE CHITIN MACROMOLECULE

Chitin is less reactive than cellulose when an attempt is made to modify it chemically, because of the characteristics of its supermolecular structure—the presence in chitin of stronger hydrogen bonds and a small active surface area, which follows from the determination of the amount of non-freezing water [90] and the measurements of the heats of swelling of chitin and cellulose [90].

In contrast to cellulose, there are only two hydroxy-groups in chitin, which can be involved in chemical reactions and can form disubstituted chitin derivatives. However, owing to their low reactivity, complete substitution is not usually achieved. The known reactions of chitin modifications are indicated schematically below:

Scheme



$R^1 = CH_3$ or $CH_3C_6H_4$; $R^2 = CH_3$ or C_2H_5.

1. The Synthesis of Esters

Chitin acetates cannot be obtained by the method [91] involving the use of perchloric acid as the catalyst. Chitin has been acetylated [92] with acetic anhydride and dry hydrogen chloride in the course of 120 h at room temperature; an overall degree of substitution of 2.99 (taking into account the acetyls group in the initial chitin) was

achieved. Chitin acetate substituted to this extent dissolves in formic acid and in 50% aqueous resorcinol solution. When water is added to the solution, acetylchitin is precipitated. Concentrated inorganic acids (sulphuric and hydrochloric) slowly dissolve acetylchitin, but it cannot be isolated from the solutions, since hydrolysis takes place. Concentrated nitric acid (specific gravity 1.50) dissolves acetylchitin very rapidly and the latter can be isolated from the solution by the addition of water, nitroacetylchitin, containing 2.14% of nitro-nitrogen, being precipitated.

Chitin has been acetylated[53] for three months with a mixture of acetic acid and acetic anhydride in the presence of zinc chloride; this yielded chitin acetate containing 2.5 acetyl groups per residue. The preparation was highly destructured.

Chitin dissolved in phosphoric acid has been acetylated [93] both in the presence and absence of perchloric acid as the catalyst. In the presence of 1% $HClO_4$, 27.5% of acetyl groups were introduced in 4 h at 75°C, which corresponded to a degree of substitution of 1.80; in the absence of the catalyst, a maximum degree of substitution of 1.70 was attained at 80°C. It is seen from these data that the limiting degree of substitution is not reached under homogeneous conditions. The esters obtained dissolved in 50% aqueous resorcinol solution and phenol and partially in *m*-cresol.

Chitin nitrates were obtained for the first time[94] by nitrating chitin with fuming nitric acid. Schorigin and Hait[95] investigated this process in detail and established that, during nitration with nitric acid alone (specific gravity 1.50), the maximum yield of chitin nitrate containing 7.51% of nitro-nitrogen, which corresponds to the substitution of 1.5 hydroxy-groups, is attained. The chitin nitrate obtained ignites at 163°C and is denitrated by sodium hydrogen sulphide in 3 h at 16°C. Its X-ray diffraction pattern indicates a fibrous structure with the same repeat period as that of chitin[81].

Among organic solvents, nitrochitin dissolves partly only in formic acid and can be isolated from solution by the addition of water. It also dissolves in sulphuric and hydrochloric acids but is not reprecipitated by the addition of water.

Chitin xanthate was described for the first time by Thor and Henderson[96-98], who impregnated chitin with a 43% solution of NaOH at 25°C for 2 h and then mixed it with crushed ice and added carbon disulphide; this resulted in the conversion of chitin into a gel and then its dissolution. The solution is unstable, chitin is deacetylated at room temperature, and chitosan xanthate is precipitated from solution. To avoid coagulation, the solution must be stored below 0°C. Chitin xanthate solutions are suitable for the formation of filaments and films[98].

Chitin sulphate esters have been described[99,100] and the authors obtained them by treating chitin with chlorosulphonic acid in different media. In pyridine[99] the sulphate formation reaction was carried out over a period of 7 h during which 14.4% of sulphur was introduced. When the reaction was carried out in dichloroethane[100] for 2 h, a sulphate ester containing 13–15% of sulphur, depending on temperature, was obtained. Chitin with this sulphur content dissolves in water. The molecular weights of all the esters were determined by the osmometric method and were found to be 14 000–17 000, depending on the conditions of synthesis[100]. In order to avoid the possible degradation of the chitin macromolecule, it has been suggested that formamide be used as a solvent for chlorosulphonic acid[101].

Methanesulphonate and toluene-*p*-sulphonate esters[102] have been obtained by treating with methanesulphonyl and toluene-*p*-sulphonyl chlorides chitin which had been subjected to preliminary activation by reprecipitation from solution in phosphoric acid in the presence of dry pyridine. The optimum conditions under which the maximum sulphur content is achieved (10.22%) during tosylation obtain in the presence of the chitin : tosyl chloride : pyridine = 1 : 10 : 50 ratios and when the reaction is carried out at room temperature for 10 days. The mono(toluene-*p*-sulphonate) ester[102] contains 8.98% of sulphur. In the preparation of the monomethanesulphonyl derivative of chitin, the optimum conditions obtain in the presence of the chitin : methanesulphonyl chloride : pyridine = 1 : 10 : 40 ratios at room temperature and when the synthesis is carried out over a period of 7 days[102], whereupon up to 12.57% of sulphur can be introduced (the monomethanesulphonate chitin ester should contain 11.4% of sulphur).

2. The Synthesis of Ethers

Methyl ethers containing 9.34% of OCH_3 group have been obtained[103] after 45 methylations of alkali-treated chitin with dimethyl sulphate. Preliminary activation of chitin with hydrochloric acid and subsequent 15 methylations in the presence of alkali made it possible to increase the content of methoxy-groups to 16.07%, which corresponds to monomethylchitin. Monomethylchitin dissolves in formic acid, swells strongly, and partly dissolves in glacial acetic acid.

Ethyl ethers containing 33.83% of OC_2H_5 groups, which correspond to a degree of substitution of 1.59, were obtained for the first time[104] by treating alkylchitin with ethyl chloride for 10 h with stepwise heating of the reaction mixture to 130°C. During the synthesis of the ether, the acetyl groups of chitin are hydrolysed. The ether contained 2.04% of free amino-groups, 2.75% of hydroxy-groups, and 4.53% of acetyl groups, which corresponds to the composition $[C_6H_7O_2(OH)_{0.34}(NH_2)_{0.70}(NHCOCH_3)_{0.21}.(OC_2H_5)_{1.59}]_n$. The molecular weight of the ether thus obtained[103], determined in ethyl methyl ketone by the method of sedimentation in an ultracentrifuge, was 66 000. Ethylated chitin forms films with a strength of 6.8–7.3 kgf mm^2 and an elongation of 15–23%.

Hydroxyethyl ethers are obtained by treating alkali-treated chitin with epoxyethane under heterogeneous[104] or homogeneous conditions[16,105]. More severe heterogeneous conditions are required for the reaction than in the case where homogeneous conditions are employed. In both cases the formation of water-soluble ethers requires a considerable excess of epoxyethane. A nitroxyethyl ether of chitin[104], containing 5.71% of nitro-nitrogen, has been obtained from the hydroxyethyl ether.

Glyceryl ethers[106] have been synthesised by treating alkali-treated chitin with glycerol monochlorohydrin or with glycidol. Treatment of chitin with glycerol monochlorohydrin did not give rise to a water-soluble ether, despite the high content of glycerol residues (up to 49%). In contrast to the initial chitin, the ether obtained

did not dissolve even in hydrochloric or phosphoric acids, which indicated the cross-linking of the ether with traces of the glycerol dichlorohydrin present in the monochlorohydrin. When glycidol was used to alkylate chitin, ethers soluble in 4 and 8% sodium hydroxide were obtained.

Carboxymethyl ethers in the form of sodium salts (Na-CMC) have been described[107-110]. The kinetics of the carboxymethylation of chitin dissolved in solutions of alkali have been investigated[108] and it has been shown that, with increase of the concentration of sodium hydrodide to 15–20% and of temperature to 20° to 30°C, the rate of reaction increases sharply. However, an increase of the reaction temperature entails the formation of a precipitate from the alkaline solution owing to the elimination of the acetyl groups and the formation of chitosan. For this reason, at the end of the carboxymethylation reaction the reaction mixture is sometimes subjected to additional acetylation with acetic anhydride at pH 10 for 2 h at room temperature[116]. In order to determine the structure of Na-CMC, it was subjected to periodate oxidation and acid hydrolysis[111]. Chromatographic analysis of the hydrolysis product showed that substitution takes place mainly at $C_{(6)}$, which is indicated also by the susceptibility of Na-CMC to periodate oxidation. During carboxymethylation[108] under heterogeneous conditions, the acetyl groups are not hydrolysed. At room temperature, the reaction proceeds most completely and a degree of substitution of 0.8–1.0 is attained. An increase of reaction temperature to 40–60°C accelerates the hydrolysis of monochloroacetic acid, so that the main reaction requires a smaller amount of the alkylating agent.

A new method has been described[110] for the preparation of carboxymethylchitin involving the preliminary activation of chitin with dimethyl sulphoxide. Carboxymethylchitin with a degree of substitution of 0.99, which is readily soluble in water, has been synthesised in this way. Acidification of Na-CMC with a dilute hydrochloric acid solution[109] yielded a chitino-glycolic acid, which swells strongly and partly dissolves in water, in contrast to celluloso-glycolic acid[112]. Na-CMC containing 6% Na and more readily dissolves in water. Aqueous solutions of Na-CMC and H-CMC exhibit polyelectrolytic properties, i.e. the intrinsic viscosity increases on diluting the solution[113].

IV. THE PREPARATION OF CHITOSAN AND ITS PROPERTIES

The preparation of chitosan from chitin has been described in numerous articles[34,84,114-117] and patents[118,119]. Gilson[114] was the first to obtain chitosan by fusing chitin with potassium hydroxide at 180–190°C; this treatment results in the hydrolysis of the *N*-acetyl groups. A patent[118] proposes milder conditions for the preparation of chitosan, involving the heating of chitin with a 40% solution of sodium hydroxide at 110°C for 4 h. The methods of preparation of chitosan have been studied in detail[116]. The author notes that the deacetylation of chitin in 40% sodium hydroxide at 135–140°C is slow; 60% of the acetyl groups are hydrolysed after 24 h. The deacetylation of chitin at 120°C in an anhydrous medium consisting of potassium hydroxide (50%), ethanol (25%), and monoethylene glycol (25%) has been described[115]. In order to achieve the required degree of deacetylation and the corresponding viscosity, the reaction is carried out for a specified time, which yields satisfactorily reproducible data[115]. The deacetylation of chitin with a mixture of potassium hydroxide and hydroxylamine is more difficult[120]. At 100°C and pH 13 approximately 70% of the acetyl groups are removed in the course of 10–20 h.

Darmon and Rudall[67] followed the changes in the infrared spectra during the deacetylation of chitin. The intensity of the carbonyl (1625 cm^{-1}) and amide (3265 and 3100 cm^{-1}) bands diminishes during the formation of chitosan, while that of the 3365 and 3445 cm^{-1} bands increases, which indicates the appearance of the NH_2 group. X-Ray diffraction studies on chitosan have shown that it has the same crystal lattice as chitin, but the degree of order in its macromolecules is smaller[64,67]. Chitosan, regenerated in the form of a film in a salt or basic form[121], gives rise to X-ray diffraction patterns characteristic of amorphous substances.

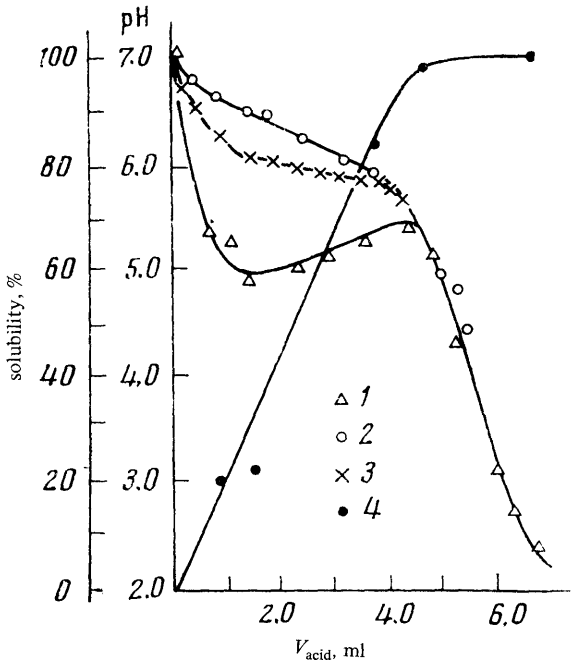


Figure 1. Potentiometric titration curves for chitosan: 1) direct titration of the initial chitosan; 2) back titration; 3) direct titration of reprecipitated chitosan; 4) solubility.

We established that the changes in the supermolecular structure during the dissolution of chitosan may be followed by its potentiometric titration. Direct potentiometric titration of the initial chitosan with acid by the individual sample method (Fig. 1, curve *1*) yields a titration curve with two extrema. The minimum at pH 4.9 corresponds to the neutralisation of the accessible amino-groups, after which further addition of acid leads to a gradual increase of pH to 5.5 (the maximum on the curve) as a result of the liberation of the amino-groups which had been previously involved in hydrogen bonding. The maximum corresponds to the complete dissolution of chitosan (Fig. 1, curve *4*); under these conditions, the supermolecular structure has been disrupted and all the

amino-groups become accessible to titration. The back-titration curve (Fig. 1, curve *2*) forms a hysteresis loop with curve *1*, from the area of which it is possible to estimate the energy consumed in the disruption of the structure [when $pH - \lg\alpha/(1-\alpha)$ is plotted against α, where α is the degree of dissociation]. This energy, amounting to 2.8 kcal mole^{-1}, corresponds to the dissociation energy of the hydrogen bonds. In the direct titration of reprecipitated chitosan (Fig. 1, curve *3*), the area of the hysteresis loop is much smaller, which indicates the incomplete restoration of the intermolecular hydrogen bonds on reprecipitation.

The viscosity and hence the molecular weight of chitosan are greatly influenced by the conditions used in the deacetylation of chitin: in an atmosphere of an inert gas (nitrogen, argon), chitosan with a higher molecular weight is obtained[122,123]. In contrast to chitin, chitosan dissolves in dilute acid solutions from which it can be isolated without alteration. Chitosan can be therefore readily characterised on the basis of its viscosity and polydispersity. A study of the flow of aqueous solutions of chitosan hydrochloride in a capillary showed[124] that its solutions do not exhibit structural viscosity.

The amino-group imparts polyelectrolytic properties to chitosan, as shown by a study of the viscosity of chitosan hydrochloride [solutions][124,125]. It was found that, at a fairly high ionic strength, the intrinsic viscosity becomes a linear function of concentration. In order to suppress completely the polyelectrolytic effect of chitosan solutions in acetic acid, it is necessary to add sodium chloride to a concentration of 0.5 M; in hydrochloric acid, this is achieved at an acid concentration of 0.3 M.

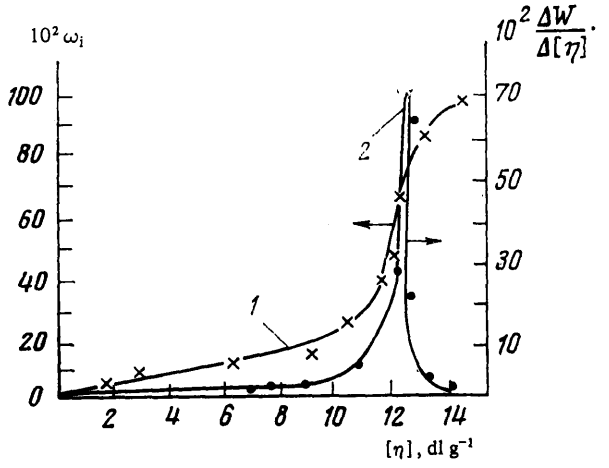


Figure 2. The integral (curve *1*) and differential (curve *2*) curves for the distribution of chitosan with respect to molecular weight; w_i is the proportion by weight of the given fraction, W the overall proportion by weight, and $[\eta]$ the intrinsic viscosity.

The polydispersity of chitosan has been demonstrated qualitatively in the extraction of chitosan lyophilised with alcohol[122], which resulted in a decrease of the relative viscosity. The molecular weight distribution has been studied by the fractional precipitation of chitosan from solution in 2% acetic acid[123] with acetone. The integral and differential distribution curves with respect to molecular weight (Fig. 2) indicate a comparative homogeneity of chitosan.

V. REACTIONS IN THE CHITOSAN CHAINS

The presence of two hydroxyls and of a primary amino-group in chitosan extends the scope of its possible modification. In the synthesis of chitosan derivatives, the preparation of specifically substituted compounds (the synthesis of *O*- or *N*-derivatives) is of special interest. The method developed previously for the specific synthesis of chitosan esters (*O*- and *N*-sulphochitosans)[126] has been recently supplemented by a method for the specific synthesis of ethers (*O*-sulphoethyl- and *O*-carboxymethyl-chitosans)[127]. The synthesis of selectively substituted chitosan derivatives ensures the formation of compounds with specified properties, required in various applications.

1. The Synthesis of Esters

The sulphonation of chitosan was achieved for the first time[128] by treatment with a mixture of sulphur trioxide and dioxide at the boiling point of the mixture. The resulting ester contained 14.8% of sulphur and exhibited anticoagulation activity *in vivo* and *in vitro*. Subsequently chlorosulphonic acid in pyridine[129], the sulphur trioxide–pyridine complex[130], and sulphuric acid[131,132] were used to sulphonate chitosan. In the last case, the formation of fully *N*-sulphonated chitosan was noted[132].

A method has been developed for the selective sulphonation of chitosan by altering the reagent and media[126]. Using the pyridine–sulphur trioxide complex as the sulphonating agent in an aqueous alkaline medium at pH 9–10, the authors obtained *N*-sulphochitosan containing one sulphamate group per glucosamine unit. Subsequent treatment of *N*-sulphochitosan with a mixture of sulphur dioxide and trioxide in the cold yielded the *O*-sulphonated derivative (75% of the hydroxy-groups were substituted). The authors note the absence of anticoagulation activity of the *N*-derivative and the high activity of this kind of the *ON*-derivative of chitosan.

The phosphorylation of chitosan has been carried out by treatment with phosphoryl chloride at 40°C for 5 h.[99] The authors do not state either the degree or site of phosphorylation.

The acetylation of chitosan with acetic anhydride at 100–135°C in a sealed tube yields a highly destructured preparation, whose properties resemble those of chitin[133,134].

The acylation of chitosan by treatment with formic acid and propionic and butyric anhydrides at 110° to 125°C leads to the formation of the corresponding *N*-derivatives of chitosan[135]. A similar type of reaction occurs when alkali-treated chitosan is treated with benzoic anhydride, as shown by the insolubility of the specimens in dilute hydrochloric acid.

The arenesulphonation of chitosan has been achieved by the reaction of benzene- and naphthalene-sulphonyl chloride with alkali-treated chitosan[135]. The derivatives contained approximately one substituent per

glucosamine unit. Benzenesulphonylchitosan is partly soluble in dilute hydrochloric acid and readily soluble in solutions of alkali in contrast to naphthalenesulphonylchitosan, which is sparingly soluble in alkali.

2. The Synthesis of Ethers

The synthesis of chitin ethers in an alkaline medium in the absence of special precautions entails a smaller or greater degree of deacetylation. For this reason, the final products are sometimes the corresponding chitosan ethers. Thus, in the synthesis of ethyl[103], carboxymethyl[107], and glyceryl[108] ethers of chitin, the degree of deacetylation is 60% and more, as a result of which the final product was called in one instance[107] carboxymethylchitosan. In view of the simultaneous occurrence of the deacetylation and alkylation processes, the resulting compound cannot be regarded as solely an *O*-derivative.

The formation of chitosan ethers with substituents located in specified positions requires the introduction of *N*-protecting groups.

The sulphoethylation of chitosan[136] has been carried out by treatment with sodium β-chloroethanesulphonate in the presence of alkali in isopropyl alcohol and in mixtures of *o*-xylene with propyl and isopropyl alcohols. The highest degree of substitution reached 0.35. Determination of the position of the substituent by the analysis of the amino-nitrogen and conductimetric titration showed that both *O*- and *N*-sulphoethylation take place.

For specific *O*-alkylation of chitosan, the amino-group was protected by reaction with an aromatic aldehyde, which results in the formation of a Schiff base[127,137]:

$$[C_6H_7O_2(OH)_2NH_2]_n + nR^1C_6H_4CHO \rightarrow [C_6H_7O_2(OH)_2N=CHC_6H_4R^1]_n ;$$
(I) (II) (III)

$$(III) \xrightarrow{ClR^2ONa} [C_6H_7O_2(OH)_{2-x}(OR^2ONa)_xN=CHC_6H_4R^1]_n ;$$
(IV)

$$(IV) \xrightarrow{CH_3COOH} [C_6H_7O_2(OH)_{2-x}(OR^2OH)_xNH_2 \cdot CH_3COOH]_n + R^1C_6H_4CHO ;$$
(V)

$$(V) \xrightarrow{NaOH} [C_6H_7O_2(OH)_{2-x}(OR^2ONa)_xNH_2]_n ;$$
(VI)

$R^1 = H, OH, OCH_3$ or NO_2; $R^2 = C_2H_4SO_2$ or CH_2CO;

x is the degree of substitution. Chitosan was treated in a neutral medium with the corresponding aromatic aldehyde at room temperature for 3 h. In contrast to benzaldehyde, anisaldehyde, and *o*-nitrobenzaldehyde, salicylaldehyde reacts fully with the amino-groups of chitosan. Salicylidenechitosan is stable in an alkaline medium but decomposes in an acidic medium into the initial components. The sulphoethylation of salicylidenechitosan has been carried out under the conditions used for the sulphoethylation of chitosan[136]. The resulting sulphoethylsalicylidenechitosan was decomposed with 50% acetic acid and then treated with a 2% solution of sodium hydroxide in order to convert it into the basic form. The final product was *O*-sulphoethylchitosan, which was confirmed by the Van Slyke analysis of the amino-nitrogen and conductimetric titration; the highest degree of substitution was 0.31. Such sulphoethylchitosan is soluble in water and in 2% acetic acid solution; when the degree of substitution is lower, the product is soluble only in dilute acids.

O-Carboxylation of chitosan has also been achieved via salicylidenechitosan[127] by treating the latter with sodium monochloroacetate; using 9 moles of the reagent per mole of the chitosan, a monosubstituted carboxymethylchitosan with free amino-groups was obtained.

Cyanoethylchitosan was obtained by the reaction of alkali-treated chitosan with acrylonitrile at different temperatures[138]. It was shown that it is desirable to carry out the reaction at 20°C for 24 h, since this entails the complete substitution of both hydroxy-groups without the hydrolysis of the cyanoethyl groups. An increase of temperature causes the hydrolysis of the cyanoethyl groups, so that one obtains derivatives with a lower degree of substitution. Cyanoethylation does not take place in a neutral medium, while in an acidic medium 0.31 of a cyanoethyl group per glucosamine unit is introduced at 20°C over a period of 24 h.

3. *N*-Alkylation of Chitosan

The *N*-methylation of chitosan has been carried out[135] by heating powdered chitosan with a tenfold excess of methyl iodide at 100°C for 6 h. A single treatment yielded mono-*N*-methylchitosan and subsequent treatment did not increase the degree of *N*-methylation. The hydriodide of mono-*N*-methylchitosan has been obtained by treating the doubly methylated chitosan with methyl iodide at 50°C for 3 h.

N-Methoxyethylchitosan has been obtained by the reaction of hydroxyethylchitosan with methyl iodide[139].

NNN-Trimethyl- and *NNN*-triethylchitosans have been synthesised by heating chitosan up to the boiling point of the corresponding alkyl iodide in absolute ethanol in the presence of organic bases[140]. Treatment with an organic base under these conditions is more effective the higher its pK_a. In the presence of triethylamine, almost fully *NNN*-trimethylated chitosan was obtained in a single treatment, which was confirmed by NMR.[140] The NMR spectrum of the resulting *NNN*-trimethylchitosan contains a signal at 6.80 p.p.m., confirming the presence of nitrogen in the tetra-substituted state. Signals corresponding to protons attached to secondary and tertiary nitrogen are absent.

NNN-Trimethyl- and *NNN*-triethyl-chitosans in salt-forms are readily soluble in water, while in the basic forms they are soluble in benzyltriethylammonium hydroxide solutions. The iodides of the quaternary bases are unstable, evolving iodine on storage. *N*-derivatives of chitosan are polyelectrolytes and their basicity increases with the degree of substitution.

VI. THE APPLICATIONS OF CHITIN, CHITOSAN, AND THEIR DERIVATIVES

There are numerous data concerning the attempts to use chitin, chitosan, and their derivatives in various branches of industry and medicine. A wide variety of the properties of the above compounds are used: their ability to form fibres and films, their capacity for ion exchange and complex formation, and their physiological activity in the absence of toxicity. The above wide

variety of properties of the chitin and chitosan compounds make it useful to synthesis new derivatives and to employ them in practice.

1. Films and Fibres Based on Chitin and Chitosan

The possibility of forming films and fibres from chitin dispersions was demonstrated as early as 1926.[10,141] However, a more thorough development of this process was achieved later in a study[97] whose authors obtained films by depositing dispersions of chitin xanthate onto a glass support with subsequent regeneration of chitin in a coagulation bath containing 40% of ammonium sulphate and 5% of sulphuric acid in water. The resulting film has a tensile strength of 9.49 kgf mm^{-2} in the dry state and 1.75 kgf mm^{-2} in the moist state. The films did not swell in water, dilute acids and alkalis, and organic solvents. This method constitutes the basis of the formation of mixed chitin-cellulose fibres via chitin and cellulose xanthates[142]. The fibres exhibit increased dyeability and have ramielike properties. The fibre obtained from partly hydrolysed chitin by the method of Thor and Henderson[97] exhibits ion-exchange properties and can be used to regenerate and purify antibiotics, aminoacids, and other organic acids[116].

Films made of chitosan and its salts are obtained from solutions in acids or water[143-145]. The films are distinguished by a high strength, resistance to alkalis, and possess effective electrotechnical properties[121,146]. When such films were acetylated by treatment with acetic acid and dicyclohexylcarbodi-imide in solutions of alkali or in water containing an organic base, chitin films were obtained with a high mechanical strength, rigidity, and transparency[147]. The regenerated chitin films retain their properties for 30 years[148].

In order to obtain chitosan fibres, Chi-Ming Pao used a solution of chitosan in acetic acid with added lead acetate, glycerol, and alcohol and a precipitation bath containing alkali, glycerol, and sodium sulphate[116,149]. The strength of this fibre corresponds to 9–10.8 dissociative km [? (Ed. of Translation)] and the elongation on rupture is 30%. A method is also available for the preparation of fibres from a mixture of aqueous solutions of chitosan and poly(vinyl alcohol)[150]. The resulting fibres can be dyed with direct acid dyes and show a low capacity for the acquisition of an electrical charge. The use of chitosan in textile industry as a finishing[151], sizing[152], and anti-shrinking agent[153], which improves at the same time the capacity of fabrics for being dyed[154], is based on its film-forming properties. Chitosan can also be used as a thickening agent in pastes for pigment printing on fabrics[155-157]. In the paper industry, chitosan is used as an adhesive[158-160]. The graft-copolymer of chitosan with 2-acrylamido-2-methylpropanesulphonic acid, acrylamide, or acrylic acid is added to paper pulp in order to improve the printing and mechanical properties of the paper[161,162]. When a chitosan coating is applied to the surface of lithographic paper[163] and photographic paper[164,165], the sensitivity of the paper to the formation of an image and the stability of the latter are improved. When chitosan is added to paper pulp and when paper is subjected to a surface treatment with chitosan solutions, many of its properties are improved: the tensile strength, breaking strength, the resistance to breaking and puncturing, and also wet strength[166]. The same effect is achieved when cyanoethylchitosan is introduced into paper or when paper is subjected to a surface treatment with cyanoethylchitosan solutions; papers of this kind also exhibit improved dielectric properties[167].

2. Anion Exchangers Based on Chitosan

The anion-exchange properties of chitosan due to the presence of the primary amino-group have aroused interest in this substance as a basis for the preparation of anion-exchange resins. Such resins have been obtained by treating chitosan with formaldehyde[168] and epichlorohydrin[169,170] or by treating chitin with epichlorohydrin and subsequent deacetylation[171,172]. The resins based on chitosan are resistant to alkalis and have an exchange capacity of approximately 4×10^{-3} mole g^{-1} (with respect to a neutral salt). These resins have been used to separate DL-mandelic acid into its optical isomers[171]. Cellulose impregnated with chitosan formate[173] and paper impregnated with chitosan[174] are used in the ion-exchange chromatography of nucleic acids. The separation on ion-exchangers of this kind is more effective than on paper impregnated with diethylaminoethylcellulose.

3. Analytical Applications of Chitin and Chitosan

Chitin and chitosan have been recently used in various types of chromatography for the separation of nucleic acid derivatives[175,176], for the isolation of egg albumen lysozyme[177,178], and for the separation of the tobacco mosaic virus[179]. Using chitin or chitosan, agar has been fractionated with respect to the degree of sulphonation[180] as a result of the formation of a complex with the highly sulphonated component of agar.

Colloidal titration of chitosan[139] and glycol-chitosan[181] has been used to determine pectin and pectic acid and colloidal titration of hydroxyethyl- and methoxyethyl-chitosan has been used to determine lignosulphonic acid[182].

The use of chitosan for the determination of heavy metals in sewage, sea water, and aqueous brines, is based on its capacity to form polymeric complexes with such metals[183-195]. Chitosan has been used as a chromatographic solid carrier for the selective extraction and accumulation of mercury, cobalt, gold, antimony, silver, chromium, iron, zinc, iridium, palladium, copper, cadmium, nickel, and lead ions[183,184,187-189,192]. The resistance of chitosan to radiation with retention of chelating properties suggested its use for the concentration of waste from nuclear fuel[185,190,191]. These studies have been reviewed in detail in a monograph[7].

4. The Applications of Chitosan and Its Derivatives in Medicine

There are numerous data concerning the usefulness of chitosan for the treatment and diagnosis of various diseases. This is promoted by the low toxicity of chitosan and its derivatives even in large doses[196].

Studies on these lines were begun by the investigation of the use of sulphochitin and sulphochitosan as blood anticoagulants. In terms of their activity, the above compounds are comparable to the natural anticoagulent heparin[197-200]. The study of the influence of the structure of sulphochitosan (*O*- and *N*-sulpho-derivatives) on its anticoagulation activity revealed that it is manifested only when all the amino-groups are sulphonated and increases rapidly with increase of the degree of *O*-sulphonation[201]. The introduction of carboxy-groups by oxidising chitosan sulphate increases its activity. In the intravenous administration of chitosan, its molecular weight is important: a decrease of the latter increases the toxicity of the

preparation[129]. By sulphonating chitosan films, antithrombogenic surfaces, retarding the coagulation of blood, have been obtained[202]. Complexes of chitosan with heparin and dextran sulphate also exhibit anticoagulation activity[203,204].

Studies of another type involve the use of chitosan for the treatment of the gastric ulcer based on its antacid activity[205] and the ability to suppress the activity of pepsin[206]. A composition containing chitosan has been proposed and has been recommended under conditions of increased acidity[207]. Chitin-based compositions accelerate the healing of wounds when applied to the affected surface[208,209]. In one study[210], the antisclerocic activity of chitosan due to the activation of lipoprotein lipase[211], was noted. The influence of chitosan on the activities of certain enzymes has been observed in a number of studies: inhibition of deoxyribonuclease[212], activation of hyaluronidase[213], and β-glucuronidase[214]. The complex salt of chitosan with the 1,4-lactone of D-glucaric acid also exhibits physiological activity[215].

Reports have appeared recently on studies of the use of chitosan for the inhibition of cell growth[212] and the decomposition of cells[216] in certain types of malignant tumours. The complex of chitosan with iododeoxycytidylic acid penetrates cancer cells selectively, which facilitates the diagnosis of cancer[217]. Chitosan causes the selective aggregation of the cells of L 1210 leukaemia *in vitro*[218].

5. Other Applications of Chitin and Chitosan

Individual reports indicate the usefulness of chitin, chitosan, and their derivatives in a wide variety of branches of the national economy. In the mining industry, chitin sulphate, carboxymethylchitin, and carboxyethylchitin are used for the preparation of drilling compositions[219] and the water-soluble salts of these derivatives are suitable for the formation of cementing materials[220]. Partly deacetylated chitin is used in the dye industry for the improvement of the susceptibility of glass[221] and synthetic[222] fibres to dyeing. Finely ground chitin is added in the cosmetic industry as an additive to creams, gels, and other cosmetic agents[223]. In the food industry, chitosan is used to clarify plant juices and extracts[224]. Chitosan has also been proposed for the coagulation of turbidity in various aqueous media[225].

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Institute of Macromolecular Compounds, USSR Academy of Sciences, Leningrad