Oxidative Destruction of Chitosan Under the Effect of Ozone and Hydrogen Peroxide

N. N. KABAL'NOVA,[1] K. YU. MURINOV,[1] I. R. MULLAGALIEV,[1] N. N. KRASNOGORSKAYA,[1] V. V. SHERESHOVETS,[1] YU. B. MONAKOV,[1] G. E. ZAIKOV[2]

[1] Institute of Organic Chemistry, Ufa Scientific Centre of Russian Academy of Sciences Russia, 450054, Ufa, Prospekt Oktyabrya, 71

[2] Institute of biochemical physics RAS, Moscow, av.Kosygin 4, Russia

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ABSTRACT: In the present work the results of the study of oxidative destruction of chitosan effected by ozone and hydrogen peroxide are listed. The results obtained verify the rupture of 1,4-β-D-glucoside bonds in macromolecule to be the basic process during amino groups of chitosan protection by acids. The rate of ozone with chitosan interaction depends on the concentration of ions in reaction mass. Destruction of chitosan by hydrogen peroxide leads to the formation of oligosaccharides in contrast to ozonolysis. Hydrogen peroxide oxidizes functional groups of chitosan only under tough conditions.
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INTRODUCTION

There are many data on the use of nitrogen-containing polysaccharide chitin (poly[1,4-2-acetamide-2-desoxy-β-D-glucose) and its deacetylated derivative-chitosan in medicine and biotechnology.[1–3] These compounds are used as medicinal preparations or their carriers (tablets, films, gels), which make it possible to prolong the effect and to decrease the toxicity of preparations.[3–5] The studies carried out during the last years showed chitin and chitosan to be promising as basic ingredients for medicinal preparations possessing antimicrobic, activity immunostimulating and antigenic effect, in particular.[6–8]

As a result, the problem to obtain oligomers of chitin and chitosan using nontoxical reagents that can be easily removed from a reaction medium is urgent.

It was shown previously that ozone and hydrogen peroxide easily depolymerize structural analog of chitosan–cellulose, and can be easily removed from a reaction medium,[9–12] which is very important for obtaining medicobiological preparations.

In the present work the results of the study of oxidative destruction of chitosan effected by ozone and hydrogen peroxide are listed.

In our experiments, chitosan obtained from Far-Eastern crabs with the initial polymerization degree P_n = 860–1100 and deacetylation degree 72–80% was used. Ozonization of polysaccharide in the form of suspension in distilled water or solutions in diluted HCl or CH_3COOH was carried out in 10–100-mL thermostated bubble reactor at 10–70°C. Ozone–oxygen mixture (2% O_3) was blown with the rate 2.4–5.4 l/h. The amount of reacted ozone was determined spectrophotometrically by measuring the concentration of O_3 in gas flow at the inlet $[O_3]_0$ and the outlet $[O_3]$ of the reactor at λ = 300 nm both for reaction media in the presence of chitosan and without polysaccharide introduction (idle experiments).[13] To con-

Correspondence to: G. E. Zaikov.

Table I Change in Molecular Characteristics of Chitosan during Ozonization of Suspension of Polysaccharide ($6.2 \cdot 10^{-2}M$) in Water at 20°C

				Elemental Composition,[c] %			
τ, min.	O_3, mmol	O_3/A,[a] mole/mole	Gel-fraction,[b] %	C	H	N	O
0	—	—	—	45.5	6.7	8.1	39.7
15	0.4	0.13	26	42.2	6.5	6.1	45.1
30	0.64	0.21	58	43.9	6.0	6.0	43.1
240	4.64	1.50	96	46.1	6.9	6.0	42.0

[a] The ratio between reacted O_3 and a total content of component units of chitosan A.
[b] Determined by solubility in $0.33M$ CH_3COOH at 25°C.
[c] Relative error is ±0.3%.

trol kinetics of O_3 consumption in reaction medium, ozone–oxygen mixture was blown through chitosan solution in a thermostated quartz cell until a necessary initial concentration was achieved. The consumption of ozone was controlled by the change in the optical density at $\lambda = 260$ nm. Reaction kinetics was studied by the change in a specific (η_{sp}) and intrinsic ($[\eta]_{sp/s}$) viscosities of acetic and muriatic solutions of chitosan.

The destruction in the presence of hydrogen peroxide under homogenous conditions (20–50°C) was studied by measuring kinematic viscosity (ν, cSt) of the solutions of chitosan in diluted acidic acid in capillary viscosimeters. In heterogeneous water medium the reaction was interrupted by the excess of acetone in a centrefuge.

Dried products were solved in acidic acid, and the kinematic viscosity of solutions obtained was measured. Characteristic viscosities were measured in Ubellode viscosimeters using $0.33M$ CH_3COOH with the addition of $0.3M$ NaCl as solvent.

Molecular mass M_n was determined by GPC on an instrument LC 1304 (eluent—$0.33M$ CH_3COOH + $0.3M$ NaCl in water). IR spectra were recorded on a spectrometer Specord, M 80, and ^{13}C NMR spectra on a spectrometer Bruker AM 300 (in HCl solution in D_2O). A content of carboxyl and amino groups was determined by potentiometric titration of neutral and muriatic water solutions or suspensions of hydrolysis products with KOH solution on an instrument pH 340.

Oxidation with Ozone: Heterogeneous Reaction

Ozone reacts with chitosan quickly enough.[14] As follows from Table I, one ozone molecule was consumed approximately on eight elemental component units of chitosan under mild conditions already in 15 min, and in 4 h the consumption of O_3 was 1.5 times as much as that of polysaccharide. Yellowing of chitosan was observed.

Proceeding of oxidative processes with the formation of acetic groups is confirmed by the appearance of a band at 1740 cm^{-1} in IR spectra, which can be attributed to valency fluctuations of a C═O bond in the carboxyl groups. The results of direct potentiometric titration verify the formation of one carboxyl group on a number of elemental component units of polysaccharide. Elemental composition of chitosan oxidized with ozone changes negligibly with the increase of the depth of ozonization (Table I).

Negligible decrease in the content of nitrogen in comparison with the initial sample is observed, which depends, probably, on deaminization.[11] At the same time, the processes proceed, which lead to lacing macromolecules, it results experimentally in the formation of gel fraction insoluble in diluted acids. The content of the latter increases, depending on the dose of ozone consumed (Table I).

The comparison of obtained results with the experiments with ozonize cellulose[9,10,12] allows supposition that lacing reactions can be mainly caused by the presence of amino groups in chitosan.[15] There are data that the stability of amines to O_3 effect increases considerably under their protonation with acids.[16] We have selected acids with various pK and anions—CH_3COOH and HCl—in which chitosan is well dissolved. The studies using gravimetry, elemental analysis, and potentiometry methods of acid salts with polysaccharide showed that practically all amino groups are protonated already in weak solutions of CH_3COOH and HCl (under the condition of a

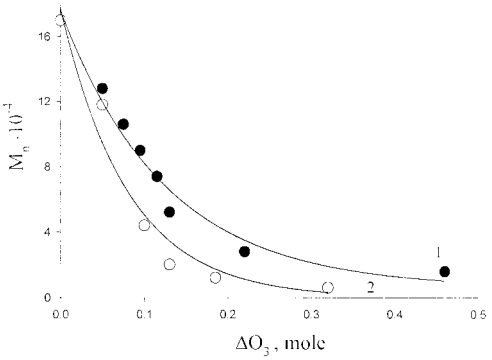


Figure 1 Dependence of numeral-average molecular mass M_n on the amount of ozone consumed O_3 (mmol) by the ozonization of chitosan in 0.1*M* HCl (1) and 0.33*M* CH_3COOH (2); $[Chitosan]_0 = 6.2 \cdot 10^{-2}M$, 20°C.

little excess of acid in relation to aminogroups). For instance, the treatment with a base of pK > 6.3 makes possible to deprotect, and washing polysaccharide precipitate with water—to remove acid. During some experiments the share of hydrolysis of chitosan under ozonization conditions was not found to be high (4–5%), which was taken into account in the calculations.

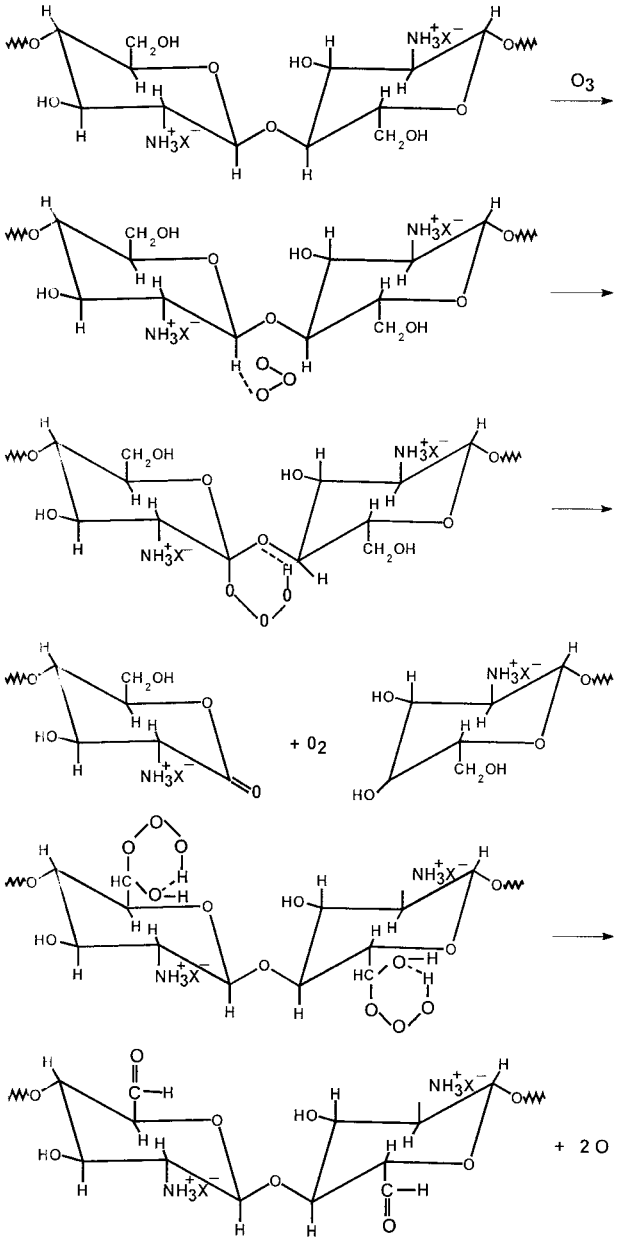
Homogeneous Reaction

Ozonization of chitosan in diluted water solutions of CH_3COOH (0.33*M*) and HCl (0.1*M*) does not lead to lacing reactions. Elemental composition, IR and ^{13}C NMR spectra of reaction products do not practically change. At the same time, molecular mass of polysaccharide decreases appreciably in proportion to reaction time or dose of ozone consumed (Fig. 1). Temperature rise increases the initial rate of destruction and decreases degree limit of polymerization (Table II).

Table II Dependence of Average Numeral Degree of Chitosan Polymerization P_n on Time and Temperature of Destruction[a]

	τ, min						
T °C	5	10	15	20	30	60	120
20	780	650	550	460	320	170	90
40	500	310	250	200	140	60	40
60	310	180	120	75	60	25	—

$[Chitosan]_0 = 6.2 \cdot 10^{-2}M$; $[HCl]_0 = 1 \cdot 10^{-1}M$.
[a] Rate of flow of ozone–oxygen mixture is 5.4 L/h.



Scheme 1

Under mild conditions elemental units of chitosan with protonated amino groups are stable enough to the oxidation with ozone. Oxidative destruction of β-D-glucoside bonds between units in macromolecules of polysaccharides is a basic reaction. In this case, degrees limit of polymerization of are in the range 30–50. According to the data[9,10] the initial stage of the interaction of ozone with polysaccharide is its electrophilic attack on C(1)—H bond with the formation of labile hydrotrioxides, destruction of which leads to depolymerization of polysaccharide (Scheme 1).

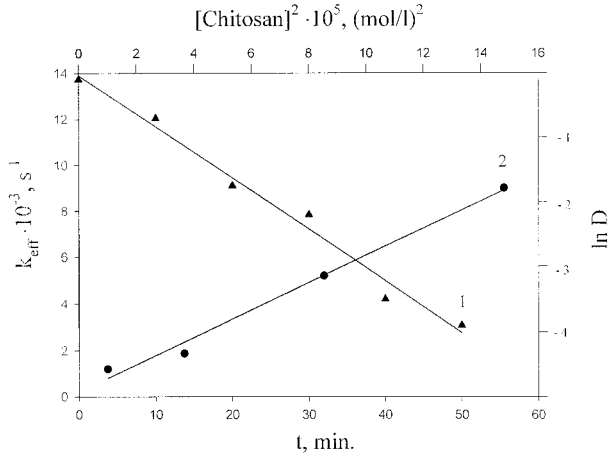


Figure 2 Semilogarithmic anamorphous of kinetic curve of the consumption of ozone in reaction with muriatic solution of chitosan (1) and dependence of k_{eff} on [Chitosan]^2 (2); [HCl] = 0,025*M,* 22°C.

Under the prolonged effect of ozone as well as high temperature (6 h, 70°C) carbonyl groups appear. Using the ^{13}C-NMR method, two carbonyl carbon atoms of carbon C═O (169.4 and 173.0 ppm) were found, which can appear both as a result of the destruction of labile hydrotrioxides (see Scheme 1) and during oxidation of the C(6)—H bond.[10]

The effect of the concentration of ozone, chitosan, and acid on the rate of the interaction of O_3 with polysaccharides was studied. It should be noted that muriatic solution of chitosan is destructed with a lower degree than the acetic one at the same doses of ozone consumed (see Fig. 1). In our opinion, it is caused by the effect of medium on the conformation of macromolecules, which, in its turn, effects the kinetics of destruction. Chitosan salts in solutions are polyelectrolytes, conformation of which are highly sensitive to the external conditions (concentration, temperature, antiions, ionic force of a medium).[17,18] We have studied kinetic regularities of the interaction of ozone with muriatic chitosan at various magnitudes of ionic force of the solution.[19]

Under the conditions when the content of HCl as well the concentration of chitosan are constant and the concentration of ozone is higher ([Chitosan]$_0 \sim (0.31 \div 1.24) \cdot 10^{-2}$ and [O_3]$_0 \sim (1.0 \div 3.7) \cdot 10^{-4}M$), the latter is a consumed pseudomolecular with a rate constant k_{eff} (Fig. 2). Under experimental conditions the idle decomposition of ozone can be neglected: rate constants of O_3 decomposition in water and in 0.025*M* HCl solution are equal to $(0.23 \div 0.09) \cdot 10^{-4}$ and $(1.22 \div 0.18) \cdot 10^{-4}$ s^{-1}, respectively.

The increase in concentration of acid in a reaction medium at constant content of chitosan leads to the decrease in k_{eff} (Fig. 3). This effect is not caused by the diminution of a number of free NH_2 groups, because amino groups are completely protonated completely under these conditions. This fact can be explained proceeding from the notion about chitosan as polyelectrolyte. According to the data,[17] macromolecules of this polysaccharide in diluted muriatic solutions have swollen ball conformation, dimensions of which for the given molecular mass are determined by various factors including the magnitude of the ionic force of the solution I_o. Polyelectrolyte effect of the unrolling of the polymer chain is caused mainly by the presence of protonated amino groups, and it is the sharper the lower the ionic force of the solution. The decrease in anionic force from 0.5 up to 0.004 increases the length of the linear segment of the macromolecule of chitosan in HCl solution two to three times. In our experiments the magnitude of I_o increases from 0.025 up to 0.1, which has to lead to rolling molecular chains of chitosan in a solution, and, consequently, to hamper the attack of ozone in solution. The diminution of the magnitudes of an intrinsic viscosity $\eta_{sp/s}$ (symbath to the change of k_{eff}) with the increase in a concentration of acid (Fig. 3) verifies the decrease in the dimension of balls.

Effective constant k_{eff} depends linearly on the temperature. Activation energy 91.4 ± 20.6 kJ/mol was found from the Arrenius dependence of k_{eff} in the range 10–40°C (at I_o = 0.025 and concentration of chitosan $1.24 \cdot 10^{-2}M$).

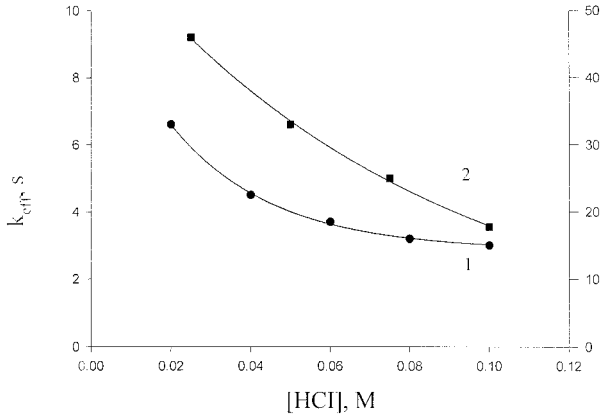


Figure 3 Dependence of k_{eff} (1) and intrinsic viscosity $\eta_{sp/s}$ (2) on concentration of acid; [Chitosan]$_0$ = 1,24 · 10*M,* 22°C.

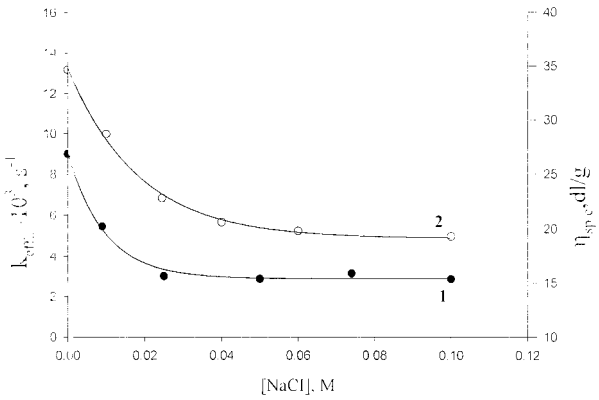


Figure 4 Dependence of k_{eff} (1) and intrinsic viscosity $\eta_{sp/s}$ (2) on concentration of NaCl; $[Chitosan]_0 = 1{,}24 \cdot 10^{-2}M$, $[HCl] = 2{,}5 \cdot 10^{-2}M$, 22°C.

Ionic force of the solution can be varied not only by changing concentration of the acid but also by the addition of another polyelectrolyte, establishing in that way the pH of the medium. To this end, sodium chloride was used, concentration of which was varied in the range $(2.5 \pm 10) \cdot 10^{-2}M$. Effective rate constant k_{eff} as well as an inrinsic viscosity of solutions at constant concentrations of HCl and chitosan decrease with the increase of NaCl content (Fig. 4). This fact testifies to the effect of conformation of macromolecules on the interaction of polysaccharide with ozone.

Thus, at constant concentration of muriatic salt of chitosan in water solutions an effective rate constant of the reaction of ozone with chitosan decreases with the increase of the concentration of electrolyte in the reaction medium. This is connected with the effect of ionic force of the solution on conformation of macromolecules of polysaccharide.

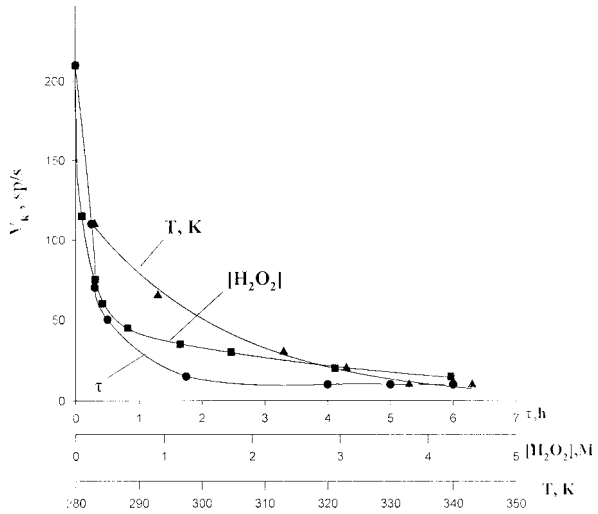


Figure 5 Dependence of destruction degree of chitosan by hydrogen peroxide on time (1), concentration of H_2O_2 (2), reaction temperature (3) in heterogeneous medium (time 30 min. (2,3), $[H_2O_2]_0 = 6.0 \cdot 10^{-1}M$ (1,2), 30°C (1,2)).

Table III Kinetic of Chitosan Destruction by Hydrogen Peroxide in Geterogeneous Medium

Time (min)	ν cSt	NH_2 Groups, %	Elemental Composition, % C	H	N	O
10	81.9	75.3	45.3	6.80	7.24	42.29
20	54.2	74.0	44.98	7.38	7.62	40.02
30	38.6	70.5	44.29	7.15	7.21	41.35
120	10.4	—	44.24	7.37	6.87	41.52
200	6.2	71.0	45.09	7.18	7.35	40.38
360	5.5	69.6	45.05	7.43	7.12	40.40

($[Chitosan]_0 = 6.2 \cdot 10^{-2}M$, $[H_2O_2]_0 = 6.0 \cdot 10^{-1}M$, 30°C, pH 7).

Oxidation by Hydrogen Peroxide

There are no literature data on kinetic regularities of the chitosan destruction under the effect of hydrogen peroxide, as well as on the effect of the phase state of medium and various factors (concentration of reagents, temperature, pH) on a reaction rate and composition of products.

In heterogeneous medium under mild conditions (pH 7, 30°C) molecular mass of polysaccharide decreases already after the beginning of the reaction (Fig. 5, curve 1).[20] Concentration of hydrogen peroxide considerably affects the rate of polysaccharide destruction (Fig. 5, curve 2). The use of a small quantity of peroxide (the ratio of elemental units of chitosan to H_2O_2 is 2 : 1) leads to the decrease in kinematic viscosity of acidic solutions of destructed chitosan twice in 30 min. Further increase of H_2O_2 concentration (higher than $6.0 \cdot 10^{-1}M$) does not effect the decrease of the molecular mass of polysaccharide. The rate of destruction considerably increases the rise of temperature. Elemental composition of chitosan does not change practically before and after destruction, but negligible deminution in the content of amino groups is observed (Table III). ^{13}C-NMR and IR spectra of initial chitosan and products of its reaction with H_2O_2 do not differ from each other.

Table IV Kinetics of Oxidative Destruction of Chitosan by Hydrogen Peroxide in Heterogeneous Medium under Tough Conditions

Time (min)	Soluble of Products in Water, %	$[\eta]_{char}$,[a] dL/g	$M_v \cdot 10^{-2}$	—COOH— Groups/100 Units of Chitosan	Elemental Composition			
					N	C	H	O
0	0	6.0	1389	0	7.66	44.8	7.01	40.5
30	32.0	0.95	229	0.49	7.01	44.3	6.36	42.4
60	51.3	0.49	120	1.18	7.05	44.1	6.20	42.6
120	66.7	0.28	68	1.53	6.97	44.0	6.09	42.9
400	85.3	—	2[b]	1.62	6.88	43.9	5.78	43.4

($[Chitosan]_0 = 6.2 \cdot 10^{-2}M$, $[H_2O_2]_0 = 3.0M$, 70°C, pH 7).
[a] pH 7, 22°C.
[b] Using the cryoscopy method.

Thus, during the interaction of chitosan with hydrogen peroxide in neutral medium under heterogeneous conditions the rupture of glycoside bonds in chitosan is supposed to be the basic reaction.

Proceeding of oxidative processes at the same time with destruction during the interaction of chitosan with hydrogen peroxide is marked under tough conditions ($[H_2O_2] = 3.0M$, 70°C). In this case elemental composition of the reaction products changes: the content of C, H, and N decreases, the content of O increases (Table IV). Carboxyl groups are also observed, their number is in proportion to the destruction depth but is no more than two groups for hundred component units of oligosaccharide.

In IR spectra the increase in the signals at 1566, 1580, and 1630 cm^{-1} is observed, which can be attributed to various oxygen containing fragments of chitosan oxidized.

The comparison of the results of the spectral as well elemental analyses, potentiometric titration of initial chitosan, and samples undergone destruction under tough conditions verifies that amino groups are the first to oxidize. During destruction of chitosan in heterogeneous medium the reactions of rupture of glucoside bonds and oxidation of some functional groups are affected not only by chemical structure but also steric configuration of macromoleculs and their reciprocal arrangement determining the availability of some chain parts.

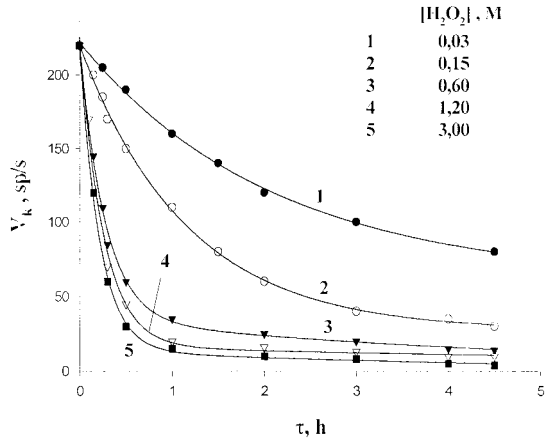


Figure 6 Kinetic curve of chitosan destruction in homogeneous medium under the effect of H_2O_2 (30°C, $[CH_3COOH] = 0.33M$, $[Chitosan]_0 = 6.2 \cdot 10^{-2}M$, $[H_2O_2]_0 = 3.0 \cdot 10^{-2}$ (1), $1.5 \cdot 10^{-1}$ (2), $6.0 \cdot 10^{-1}$ (3), 1.2 (4), and $3.0M$ (5)).

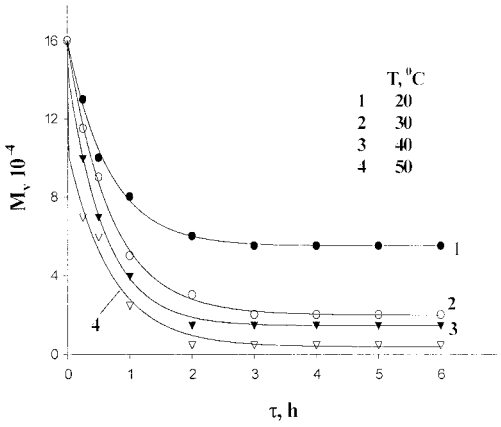


Figure 7 Kinetic curve of chitosan destruction in homogeneous medium under the effect on the reaction temperature ($[CH_3COOH] = 0.33M$, $[Chitosan]_0 = 6.2 \cdot 10^{-2}M$, $[H_2O_2]_0 = 6.0 \cdot 10^{-1}M$, T = 20 (1), 30 (2), 40 (3), 50°C (4)).

Oxidation by Hydrogen Peroxide in Homogeneous Medium

The increase in concentration of H_2O_2 and chitosan in reaction medium leads to a more rapid decrease in the chitosan polymerization degree (Fig. 6). The temperature rise of the reaction also accelerates the process.

The fact should be noted that destruction rate in acidic homogeneous medium is not much higher than that in the heterogeneous neutral one. Elemental composition ^{13}C-NMR and IR spectra of the products of homogeneous destruction do not change. In this case, the interaction with hydrogen peroxide is supposed to be also a rupture of glucoside bonds in polysaccharide macromolecules.

Thus, the results obtained verify the rupture of 1,4-β-D-glucoside bonds in macromolecule to be the basic process during amino groups of chitosan protection by acids. Polymerization degree of products can be regulated by varying the quantity of oxidant and the temperature in a wide range.

The rate of ozone with chitosan interaction depends on the concentration of ions in reaction mass, which is connected with the change in conformation of polysaccharide macromolecules.

Ozonation of water suspension of chitosan results in oxidation of amino groups accompanied by deamination and lacing macromolecules. Destruction of chitosan by hydrogen peroxide both in homogeneous and heterogeneous medium leads to the formation of oligosaccharides in contrast to ozonolysis. Hydrogen peroxide oxidizes functional groups of chitosan, in the first place amino groups, only under tough conditions.

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