## Oxidative Destruction of Chitosan During Ozonization

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Ozonization of water suspension of chitosan leads to oxidation of amino groups which accompanied by deamination and crosslinkihg of macromolecules. The clearing of $1,4$-$\beta$-$\nu$-glucoside bonds in macromolecule is a basic process in the protection of amino groups by protonation with acid. The polymerization degree of ozonolysis products in acidic medium can be varied in wide ranges depending on reaction conditions. The rate of the interaction of ozone with chitosan depends on the conformation of polysaccharide macromolecules.

*Keywords*: Chitosan; Ozone; Destruction; Oligosaccharide; Polysaccharide

## INTRODUCTION

There are many data on the use of the nitrogen containing polysaccharide chitin (poly[1,4-2-acetamide-2-desoxy-$\beta$-$D$-glucose) and its deacetylated derivative – chitosan in medicine and biotechnology [1–3]. The compounds are used as medicinal preparations or as their carriers (tablets, films, gels) which makes possible the prolongation of the effect and a decrease of the toxicity of the preparations [3–5]. Studies carried out during recent years showed the prospects of oligomers of chitin and chitosan as basic ingredients for medicinal

preparations with antimicrobical activity, immunostimulating and antigenic effect [6–8].

In view of that, a method is urgently needed to obtain oligomers of chitin and chitosan using nontoxic, easily removable reagents.

Previously it was shown that ozone depolymerizes a structural analog of chitosan – cellulose and can be easily removed from a reaction medium [9–10], that is very important for obtaining medico-biological preparations.

Results of a study of the oxidative destruction of chitosan during ozonization are described in the present work.

## EXPERIMENTAL

In this work chitosan obtained from Far-Eastern crabs with an initial degree of polymerization $P_n = 1100$ and degree of deacetylation 75% was used. Ozonization of the polysaccharide as a suspension in water and solutions in diluted HCl or $CH_3COOH$ was carried out in a 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 by spectrophotometric measurement of the concentration of $O_3$ at the inlet and the outlet of the reactor ($\lambda = 300$ nm) both for reaction media in the presence of chitosan and without the introduction of polysaccharide (blank experiments). To control the kinetics of the consumption of $O_3$ in a reaction medium ozone – oxygen mixture was blown through chitosan solution in 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. Kinetics of destruction were studied by the change in a specific ($\eta_{sp}$) and intrinsic ($\eta_{sp/s}$) viscosities of acetic and HCl solutions of chitosan. Molecular mass $M_n$ was determined by GPC on an LC 1304 instrument (eluent is 0.33 M $CH_3COOH + 0.3$ M NaCl in water). IR spectra were recorded on a spectrometer Specord – M80, $^{13}C$ NMR spectra – on a Bruker AM 300 spectrometer (in a solution of HCl in $D_2O$). The content of carboxyl groups was determined by a direct potentiometric titration of neutral water suspension of ozonolysis products with KOH solution and a pH buffer.

## RESULTS AND DISCUSSION

Ozone reacts with chitosan quickly. The data listed in Table I shows that based on elemental composition of the units of chitosan, chitosan molecule was consumed under mild conditions approximately already in 15 min, but in 4 hours the consumption of $O_3$ was 1.5 times as much than that of the polysaccharide. Yellowing of the chitosan was observed in proportion to the progress of the reaction.

Probness of the 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 the $C=O$ bond in carboxyl groups. The results of direct potentiometric titration indicate the formation of one carboxyl group per several dozens of elementary component units of the polysaccharide. Elemental composition of chitosan oxidized with ozone changes negligibly with increasing degree of ozonization (Tab. I). A negligible decrease in the content of nitrogen is observed in comparison with the initial sample, that probably depends, on deamination [11]. Together with the degradation of macromolecules, expirements show that process leading to crosslinking take place, resulting in the formation of chitosan gel fraction which is insoluble in dilute acids. The content of gel fraction increases depending on the dose of ozone consumed (Tab. I).

The comparison of the obtained results with experiments to ozonize cellulose [9, 10, 12] makes the supposition possible that crosslinking reactions in general depend on the presence of amino groups in chitosan [13]. There are data that the resistance of amines to the effects

TABLE I  Changes in molecular characteristics of chitosan during ozonization of 1% suspension of polysaccharide in water at 20°C

| $\tau$, min | $O_3$, Mmole | $O_3/A^*$, mole/mole | Gel-fraction**, % | Elemental composition***, % | | | |
|---|---|---|---|---|---|---|---|
| | | | | 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 |

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

of $O_3$ increases considerably when they are protonated by acids [14]. We have chosen acids with various pK and anions – $CH_3COOH$ and HCl, in which chitosan dissolves well. Studies using the methods of gravimetry, element analysis and potentiometric titration of acid salts separated by polysaccharide showed that practically all the amino groups of chitosan were protonated (under the conditions of a slight acid excess relative to amino groups). Treatment with base with pK > 6.3 makes possible to remove the protection, and washing of the polysaccharide residue with water leads to the removal of auxiliary reagents from the ozonolysis products. During some experiments it was established that the level of hydrolysis of chitosan in the given acids is not high under the conditions studied (4–5%). That was taken into account in the calculations.

   Subsequent experiments showed that crosslinking reactions did not proceed during ozonization of chitosan in dilute aqueous solutions of $CH_3COOH$ (0.33 M) and HCl (0.1 M), elemental composition, IR- and 13C spectra of the reaction products practically do not change. At the same time the molecular mass of polysaccharide decreases appreciably in proportion to the reaction time or dose of ozone consumed (Fig. 1).
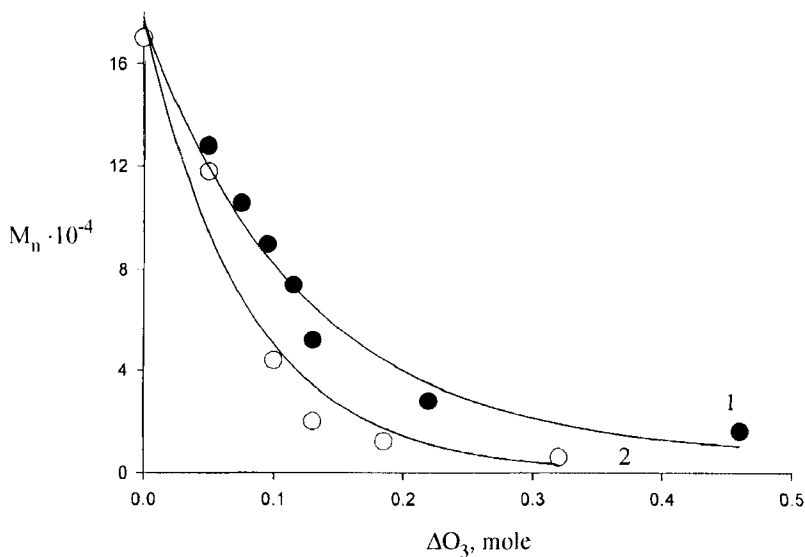
FIGURE 1   Dependence of number-average molecular mass $M_n$ on the amount of ozone consumed $O_3$ (mmole) by the ozonization of chitosan in 0,1 M HCl (1) and 0,33 M $CH_3COOH$ (2); [Chitosan]$_0$ = 6,2 · 10$^{-2}$ M, 20°C.

When the temperature raises the initial rate of destruction increases but the limiting degree of polymerization decreases (Tab. II).

Kinetic curves of destruction can be formally described as follows

$$\ln(P_n - 1)/P_n = \ln(P_n^0 - 1)/P_n^0 - k\tau$$

where $P_n^0$ and $P_n$ are average degrees of polymerization at the initial and time moments $\tau$, respectively, $k$ – is overall-constant of destruction rate (Fig. 2). In contrast to the destruction of chitosan, the kinetic curves of heterogenic destruction of cellulose consist of two plots [10], that are associated with the presence of amorphous as well more dense

TABLE II   Dependence of average numeral degree of chitosan polymerization $P_n$ on time and temperature [Chitosan] $= 6.2 \cdot 10^{-2}$ M; [HCl] $= 1 \cdot 10^{-1}$ M

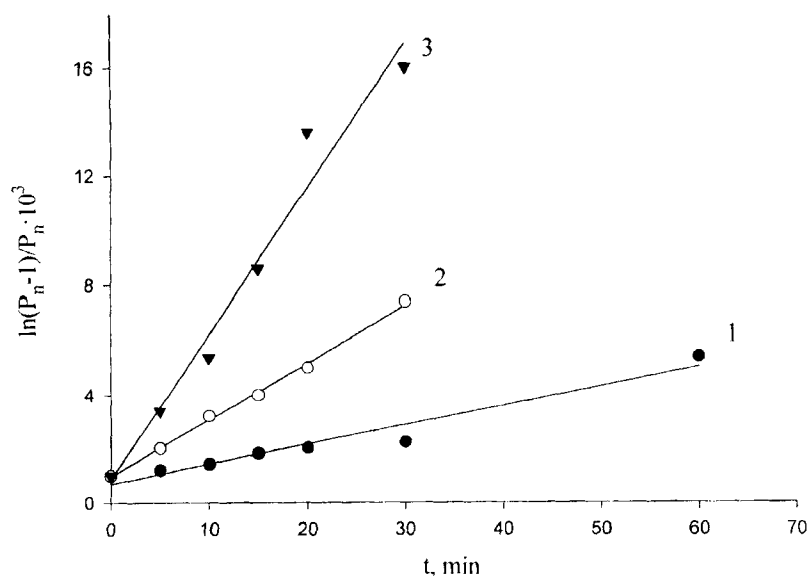| $T$, | $\tau$, min | | | | | | |
|---|---|---|---|---|---|---|---|
| °C | 5 | 10 | 15 | 20 | 30 | 60 | 120 |
| 20 | 780 | 650 | 550 | 460 | 320 | 170 | 90 |
| 40 | 500 | 310 | 250 | 200 | 140 | 60 | 40 |
| 60 | 310 | 180 | 120 | 75 | 60 | 25 | – |



FIGURE 2   Dependence of $I_n(P_n - 1)$ on ozonization time at different temperatures in 1 M HCl; Temperature (°C) – 20 (1), 40 (2), 60 (3); [Chitosan]$_0$ = 6,2 · 10$^{-2}$ M.

crystal phases of polymer. The absence of breaks in the kinetic curves during chitosan ozonolysis must be caused by homogeneous character of the process (Fig. 2). This circumstance can be one of the causes of more low limiting polymerization degrees and higher constant (approximately by one order) of chitosan destruction under the interaction with ozone in comparison with cellulose. According to Figure 2 effective overall-constants are equal to:

| $T(°C)$ | 20 | 40 | 60 |
|---|---|---|---|
| $k10^6(c^{-1})$ | $1,39 \pm 0,15$ | $3,49 \pm 0,38$ | $9,15 \pm 3,5$ |

An other factor which affects a different reactivity of chitosan and cellulose must be a difference in the structure of the macromolecules of polysaccharides. Effective activation energy of destruction of chitosan in the temperature range 20–60°C equals 38 kJ/mol ($r = 0,994$), that is close to the activation energy during the interaction of ozone with acetals [15].

Under mild conditions, elemental units of chitosan with protonated aminogroups are stable enough against oxidation with ozone. The basic reaction affects the $\beta$-$D$-glucoside linkages between elemental component units in the polysaccharide chains. In this case, the limiting degrees of polymerization are in the range 30–50. According to literature works [9, 10], an initial stage of the interaction of ozone with polysaccharide is its electrophilic attack on C(1) —H bond with the formation of labile hydrotrioxides, the destruction of which leads to depolymerization of the polysaccharide (see a scheme).

A long exposure time to the effect of ozone as well high temperatures lead to the formation of carbonyl groups (6hs, 70°C). Using $^{13}$C NMR method the presence of two carbonyl atoms of carbon C=O (169,4 and 173,0 ppm) was detected. Carbonyl groups can appear both as a result of the destruction of labile hydrotrioxides (scheme) and during oxidation of C(6) —H bond [10].

The effect was studied of the concentration of ozone, of chitosan and of acid on the rate of the interaction of $O_3$ with polysaccharides. It should be noted that HCl solution of chitosan undergoes less destruction than acetic solution under the same doses of ozone consumed (Fig. 1). In our opinion, the most obvious cause is the effect of a medium on the conformation of macromolecules, that, in turn,

affects the kinetics of destruction. Chitosan salts in solutions are polyelectrolytes, the conformation of which are highly sensitive to the external conditions (concentration, temperature, counter ions, ionic force of the medium) [16, 17]. The kinetic regularities of the interaction of ozone with HCl chitosan at different magnitudes of ionic force of a solution were studied.

Under conditions when the content of HCl as well as the concentration of chitosan are constant, and the concentration of ozone is higher $(0,31 \div 1,24) \cdot 10^{-2}$ and $(1,0 \div 3,7) \cdot 10^{-4}$ M, respectively, the ozone is consumed pseudomolecular with a rate constant $k_{eff}$. (Fig. 3). Under the experimental conditions the self 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.

A second order of the reaction on polysaccharide results from a linear dependence of $k_{eff}$ on [Chitosan]$^2$ (Fig. 3). Therefore, the rate of
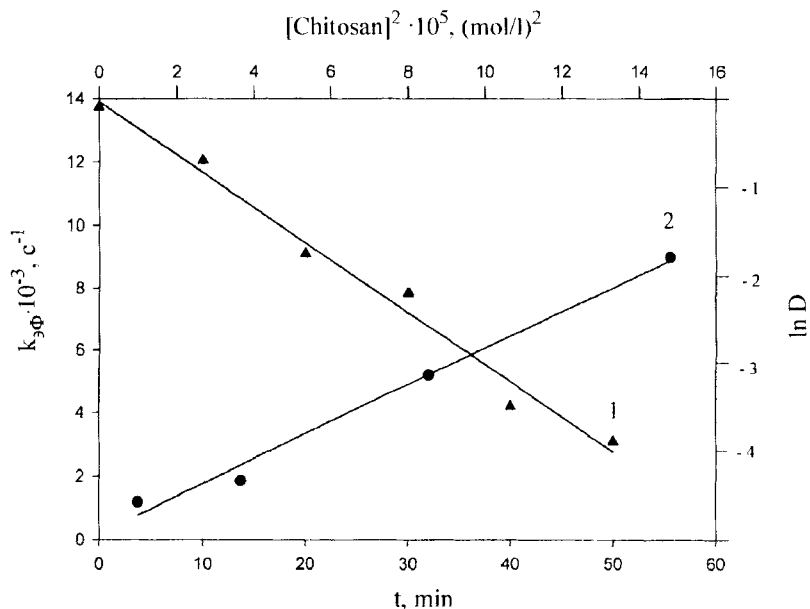
FIGURE 3  Semilogarithmic dependence of kinetic curve of the consumption of ozone in reaction with HCl solution of chitosan (1) and dependence of $k_{eff}$ on [Chitosan]$^2$ (2); [HCl] = 0,025 M, 22°C.

the consumption of ozone at constant concentration of HCl is equal
to:

$$-dO_3/d\tau = k_{eff}[O_3][\text{Chitosan}]^2$$

The increase in the concentration of acid in a reaction medium at
constant content of chitosan leads to the decrease in $k_{eff}$ (Fig. 4).

This effect is not caused by the diminuation of a number of free
$NH_2$-groups, since amino groups are protonated completely under the
conditions mentioned. This fact can be explained as arising from the
notion about chitosan as a polyelectrolyte. According to the work [16],
the conformation of macromolecules of this polysaccharide in dilute
HCl solutions have in the form of a swollen coil; the dimensions of
which for the given molecular mass are determined by various factors
including a magnitude of the ionic force of a solution $I_o$. Polyelec-
trolyte effect of the uncoiling of polymer chain is caused in the first
place by the presence of protonated amino groups, it is sharper the
lower the ionic force of the solution. The decrease in ionic force from
0,5 up 0,004 leads to two-to three fold increase in the length of a
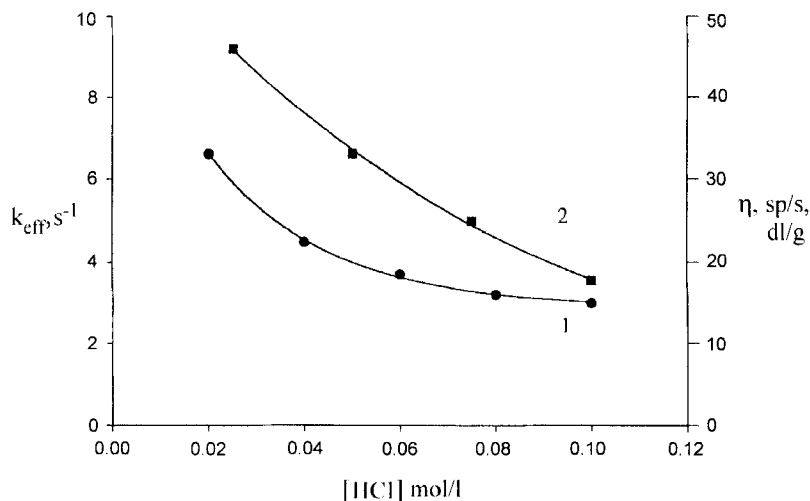linear segment of macromolecule of chitosan in HCl solution. In our



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

experiments the value of $I_o$ increases from 0,025 up 0,1 that must lead to the coiling of the molecular chains of chitosan in a solution, and, consequently, to hamper the attack of ozone. The diminution of the values of an intrinsic viscosity $\eta_{sp/s}$ (similar to the change of $k_{eff}$) with the increase in acid concentration (Fig. 4) atests to the decrease in the dimension of the coils.

The effective constant $k_{eff}$ depends linearly on the temperature. Activation energy of $91,4 \pm 20,6$ kJ/mol (at $I_o = 0,025$ and concentration of chitosan $1,24 \cdot 10^{-2}$ M) was found from Arrenius dependence of $k_{eff}$ in the range $10-40°C$.

The ionic force of a solution can be varied not only by the change of acid concentration but also by the addition of an other polyelectrolyte while maintaining the pH of the medium constant. For this purpose sodium chloride was used, the concentration of which was varied in the range $(2,5 \pm 10) \cdot 10^{-2}$ M. $k_{eff}$ and the inrinsic viscosity of solutions at constant concentrations of HCl and chitosan decreased with the increase in the content of NaCl (Fig. 5). This fact demonstrates the effect of the conformation of macromolecules on the interaction of the polysaccharide with ozone.

Thus, at constant content of hydrochloride salt of chitosan in water solutions, the effective rate constant of the consumption of ozone
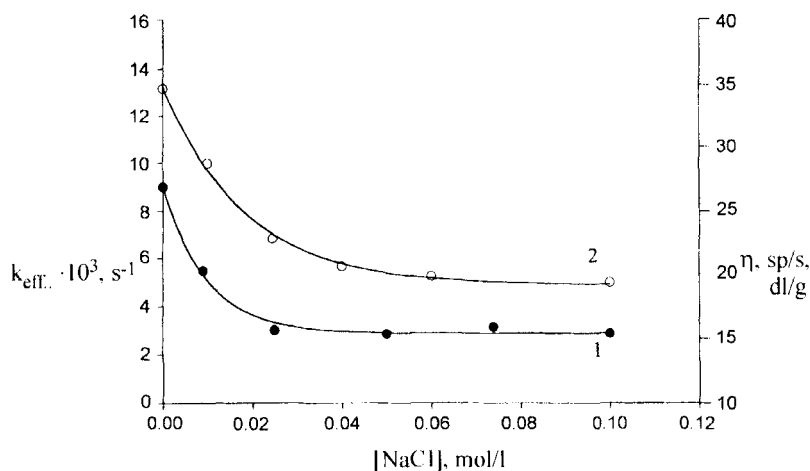


FIGURE 5  Dependence of $k_{eff}$ (1) and intrinsic viscosity $\eta_{sp/s}$ (2) on concentration of NaCl; $[Chitosan]_0 = 1,24 \cdot 10^{-2}$ M, $[HCl] = 2,5 \cdot 10^{-2}$ M, $22°C$.

decreases with the increase in concentration of electrolytes in the re-action medium. This is associated with the effect of the ionic force of the solution on the conformation of polysaccharide macromolecules.

## CONCLUSION

1. Ozonization of water suspension of chitosan leads to oxidation of amino groups which accompanied by deamination and crosslink-ing of macromolecules.
2. The clearing of 1,4-$\beta$-$\nu$-glucoside bonds in macromolecule is a basic process in the protection of amino groups by protonation with acid. The polymerization degree of ozonolysis products in acidic medium can be varied in wide ranges depending on reaction conditions.
3. The rate of the interaction of ozone with chitosan depends on the conformation of polysaccharide macromolecules.

### *References*

[1] Teslenko, A. I. (1982). *Chitin and its Derivatives: Review of information DSTI microbiological industry*, 90 p.
[2] Plisko, E. A., Nud'ga, L. A. and Danilov, S. N. (1977). Uspehi himii (Advantages of Chemistry), **46**(8), 1470–1487.
[3] Muzzarelli, R. A. A., *Chitin* Oxford: Pergamon Press, 1977, 205 p.
[4] Dubinskaya, A. M. and Dobrotvorsky, A. E. (1989). Chimiko – pharmatseftichesky Jurnal (Journal of Chemical Pharmacy), No. 5, pp. 623–628.
[5] Peter, M. G. (1995). *J.M.S. Pure Appl. Chem.*, **32**A(4), 629–640.
[6] *Patent 59-27827*, (Japan) Antimicrobe Prepar.
[7] *Patent 62-61927* (Japan) Adjuvant for the Immunostimulations Prepars.
[8] Suzuki, K., Mikati, T., Okawa, J. *et al.* (1986). *Carbohydr. Res.*, **151**(2), 403–408.
[9] Sixtra, H., Gotzinger, G., Schrittwieser, A. and Hendel, P. (1991). Das Papier, **45**, 610–614.
[10] Demin, V. A., Kabal'nova, N. N., Osipova, G. I. and Shereshovetz, V. V. (1993). *Russian Journal of Applied Chemistry*, **66**(11), 2562–2567.
[11] Langlais, B., Rechhow, D. A. and Brink, D. R. (1991). *Ozone in Water Treatment: Application and Engineering.* AWWA Research Foundation, p. 50.
[12] Mester, L. (1959). *Adv. Chem. Cer. 21. Ozon. Chem. a. Technol.*, p. 195.
[13] Razumovsky, S. D. and Zaikov, G. E. (1974). *Ozone and its Reactions with Organic Compounds.* Moscow: Science, 322 p.
[14] Pryor, W. A., Giamalva, D. H. and Church, D. F. (1984). *J. Amer. Chem. Soc.*, **106**(23), 7094–7100.
[15] Deslonglhams, P., Atlani, P., Frenei, D. *et al.* (1970). *Canad. J. Chem.*, **48**(4), 3651–3664.
[16] Tsvetkov, V. N., Eskin, V. E. and Frenke', S. I. (1984). *Structure of Macromolecules in the Solutions*, Moscow: Science, 554 p.