ISSN 1070-4272. Russian Journal of Applied Chemistry, 2006, Vol. 79, No. 5, pp. 845–849. © Pleiades Publishing, Inc., 2006.
Original Russian Text © E.N. Fedoseeva, L.A. Smirnova, M.A. Sorokina, M.O. Pastukhov, 2006, published in Zhurnal Prikladnoi Khimii, 2006, Vol. 79, No. 5, pp. 857–861.

MACROMOLECULAR CHEMISTRY AND POLYMERIC MATERIALS

Radical Degradation of Chitosan under the Action of a Redox System

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Received August 1, 2005

Abstract—Degradation of chitosan in solution under the action of redox radical initiators was studied with the aim to prepare chemically oligomers of natural polysaccharides. The influence of temperature on the rate and extent of the process was examined. The optimal ratios of the substrate to initiator and of the reductant to oxidant in the initiating system were determined.

DOI: 10.1134/S1070427206050296

Chitosan (β-1,4-bonded 2-deoxy-2-amino-*D*-glucopyranose) is the deacetylation product of chitin, a natural polysaccharide. Chitosan is a structural analog of cellulose; it has unique properties. In contrast to cellulose, chitosan is soluble in dilute aqueous solutions of some mineral and organic acids. Chitosan is widely used today in biology, cosmetics, paper production, and treatment of wastewater containing heavy metals [1]. The application fields of chitosan and its derivatives will expand because, firstly, chitin, which is the natural source of chitosan, is a renewable raw material. Chitin is present in crustacean processing wastes; it can also be recovered from insects (dead bees, mulberry silkworm, etc.), mushrooms, segmented worms, and mollusks [2, 3]. Secondly, chitosan is biodegradable, i.e., it can be utilized without environmental pollution. The molecular weight of chitosan recovered from various natural sources is usually in the range 10^5–10^6. A decrease in the molecular weight of chitosan to values typical of oligomers would expand its application field, because chitosan oligomers exhibit certain specific properties untypical of high-molecular-weight products [4]. Chitosan oligomers dissolve in water at neutral pH values.

To obtain a low-molecular-weight product, the starting chitosan is subjected either to chemical hydrolysis or to enzymatic degradation [5–8]. However, the enzymatic degradation requires thorough purification of the raw material to remove impurities that diminish the activity of enzymes (in particular, calcium) and of the final product to remove the enzymes. Chemical hydrolysis is most frequently performed with aqueous solutions of hydrogen peroxide of various concentrations. Other reagents such as potassium persulfate [8] or ozone [5] are also used; however, in this case the purification of the oligochitosan obtained may be labor-consuming. Hydrogen peroxide seems to be preferable as degradation agent, because products of its decomposition are nontoxic and excess reagent can be readily removed from the reaction medium. However, low-molecular-weight chitosan and its oligomers are prepared with these reagents, as a rule, at elevated temperatures (50–70°C). Under these conditions, the chemical composition of the polysaccharide may change. In particular, Galiaskarova [5] observed deamination of chitosan and oxidation of hydroxy groups to carboxy groups. At lower temperatures and reagent concentrations, water-soluble chitosan is not formed.

Hsu et al. [8] proved the radical mechanism of the chemical degradation of chitosan chains under the action of reagents capable of radical generation under various conditions. The radical mechanism of the degradation in the presence of free radical initiators suggests the suitability of redox initiators. Such initiators allow the reactions to be performed at lower temperatures; the probability of undesirable transformations of chitosan functional groups can be thus decreased. It is known [9] that the hydrogen peroxide–ascorbic acid redox system efficiently initiates reactions performed at room or even lower temperature.

The goal of this study was the preparation of chitosan oligomers at low temperatures by the degradation of chitosan under the action of the hydrogen peroxide–ascorbic acid redox system. The choice of ascorbic

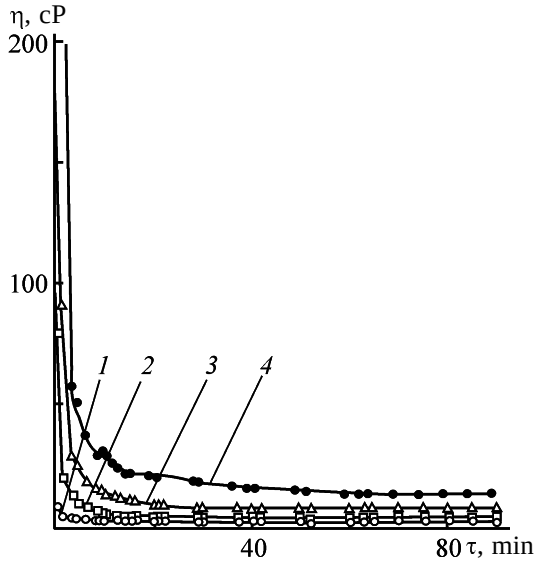


Fig. 1. Viscosity η of chitosan solutions of various concentrations as a function of the degradation time τ in 3 wt % acetic acid solution. Molar ratios: chitosan : H_2O_2 20 and H_2O_2 : ascorbic acid 1; 21°C. Chitosan concentration, wt %: (*1*) 2, (*2*) 3, (*3*) 4, and (*4*) 6.

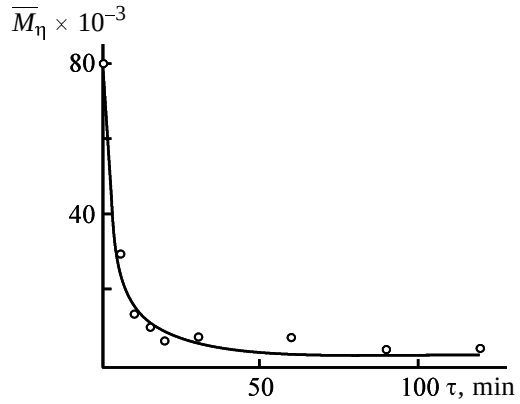


Fig. 2. Molecular weight of chitosan $\overline{M}_\eta$ as a function of the degradation time τ. Concentrations, wt %: chitosan 3 and acetic acid 1.5; molar ratios: chitosan : H_2O_2 10 and H_2O_2 : ascorbic acid 1; 21°C.

acid is governed by its efficiency as reductant [9] and by the nontoxicity of ascorbic acid and its oxidation products [10].

EXPERIMENTAL

Chitosan samples with M 8.0×10^4 and 9.6×10^4 (Sonat, Moscow) were used without additional purification (degree of deacetylation 82%, weight fraction of insoluble substances 0.25%, weight fraction of dry residue after calcination 0.3%). Ascorbic acid was of chemically pure grade, and hydrogen peroxide (30% aqueous solution), of analytically pure grade; 0.1 N HCl solution was prepared from the titrimetric standard. The solvent was prepared using chemically pure grade glacial acetic acid.

The molecular weight of samples of chitosan and its oligomers was determined viscometrically with an Ubbelohde viscometer at 21°C in an aqueous solution containing 0.33 M acetic acid and 0.3 M NaCl. The viscosity-average molecular weight was calculated by the Mark–Houwink equation $[\eta] = k\overline{M}_\eta^\alpha$, $k = 3.41 \times 10^{-5}$, $\alpha = 1.01$ [11].

The degree of deacetylation of chitosan was determined by potentiometric titration of its solutions in 0.1 N HCl with a 0.1 N solution of KOH, using an EV-74 pH meter.

The initial concentration of the hydrogen peroxide solution was determined by volumetric titration with a potassium permanganate solution in the presence of sulfuric acid [12].

To perform the chitosan degradation under the action of the redox system, we added the required weighed portion of ascorbic acid to a chitosan solution of the required concentration. After the acid dissolved, the required amount of a hydrogen peroxide solution in distilled water was added. The reaction progress was monitored by measuring the viscosity of chitosan solutions with an Ubbelohde (for dilute solutions) or Hoeppler (for moderately concentrated solutions) viscometer. The total extent of degradation was evaluated from the change in the molecular weight of chitosan after performing the reaction for 24 h.

The chemical composition of chitosan was determined by Fourier IR spectroscopy, potentiometric titration, and analysis for nitrogen (Kjeldahl method).

In this study we examined the performance of redox systems in the degradation of the chitosan backbone in solutions with pH 4.5–5.5 and the influence of the temperature and component ratio on the rate and extent of the process.

Figure 1 shows how the viscosity of chitosan solutions of different concentrations varies with time in the presence of the hydrogen peroxide–ascorbic acid redox system. The relative and dynamic viscosities of solutions (Fig. 1) and the molecular weight of chitosan (Fig. 2) sharply decrease in the first 10 min; within 15–20 min, the curves flatten out. Thus, the chosen redox system efficiently breaks the chitosan chains. Measurements of the molecular weight of chitosan as a function of the reaction time (a typical curve is shown in Fig. 2) demonstrate that the kinetic curves for the viscosity and molecular weight fully correlate

with each other, i.e., a decrease in the solution viscosity is due to a decrease in the molecular weight of the starting chitosan.

The nitrogen content in the initial and final products, determined by the Kjeldahl method, is the same (8%), which shows that the reaction does not involve chitosan amino groups. This conclusion is also confirmed by the titration results. The degree of deacetylation was 82% for all the samples.

The Fourier IR spectra of chitosan before and after the reaction are somewhat different. A new band at 1667 cm^{-1}, assigned to the stretching vibrations of the ketone carbonyl group, appears in the absorption spectrum of the low-molecular-weight product.

To determine how the extent of the decrease in the molecular weight of chitosan depends on the ratio of the redox system components, we varied the amount of ascorbic acid relative to hydrogen peroxide at a fixed ratio of the other components. Figure 2 shows that it is most appropriate to take the redox system components in an equimolar ratio. Excess ascorbic acid relative to hydrogen peroxide does not cause further changes in the molecular weight of chitosan relative to the equimolar ratio. Thus, in our further experiments we chose the equimolar ratio of H_2O_2 to ascorbic acid as the optimum.

There is also a certain limiting ratio between the substrate and initiating system (with equimolar ratio of the components) (Fig. 4). It could be expected that the degree of polymerization of chitosan would gradually decrease with an increase in the amount of hydrogen peroxide per chitosan molecule in solution; however, this is not the case. Figure 4 shows that there is a saturation plateau whose onset corresponds to approximately 0.1 mol of hydrogen peroxide per mole of the glucosamine units. Further addition of hydrogen peroxide (and, correspondingly, ascorbic acid) is virtually inefficient. Theoretically, this amount of H_2O_2 should lead to the formation of an oligomer with the degree of polymerization of 10–11. The calculated curve (Fig. 4, curve *2*) shows that actually the efficiency of utilization of hydrogen peroxide as a source of radicals breaking the chitosan backbones is appreciably less than unity (about 50%), i.e., H_2O_2 is also consumed in side reactions.

The decreased efficiency of radical initiation was also observed by Hsu et al. [8], who performed radical degradation of chitosan under the action of potassium persulfate. They considered as one of the possible reasons the deactivation of the generated free radicals in their reaction with the carbonyl groups of the arising terminal units of the oligomer. Hsu et al. believe that this reaction is responsible for the rapid reaction completion (within 1 h), compared to the half-life of the initiator at the reaction temperature (20.9 h).

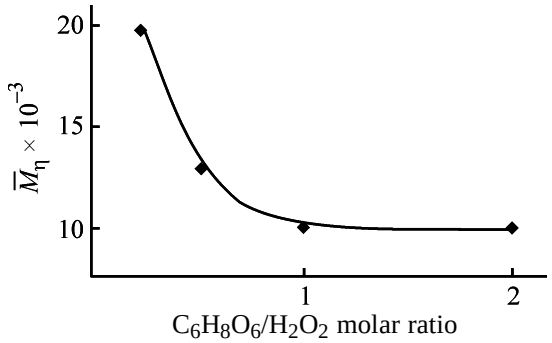


Fig. 3. Molecular weight of chitosan $\overline{M}_\eta$ measured 24 h after adding the redox system as a function of the molar ratio of ascorbic acid to hydrogen peroxide. Concentrations, wt %: chitosan 3 and acetic acid 2; molar ratio chitosan : H_2O_2 10; 21°C.

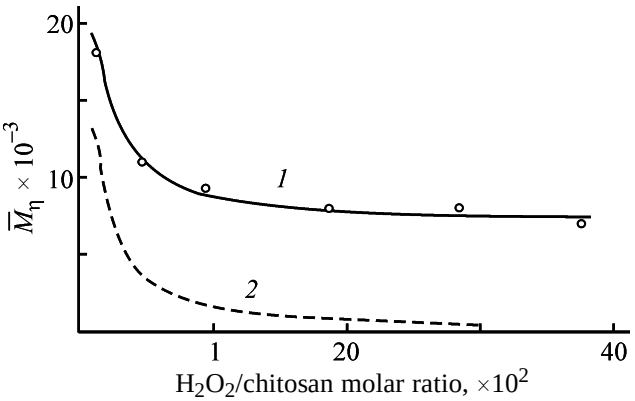


Fig. 4. Molecular weight of chitosan $\overline{M}_\eta$ measured 24 h after adding the redox system as a function of the molar ratio of hydrogen peroxide to chitosan. Concentrations, wt %: chitosan 3 and acetic acid 2; molar ratio H_2O_2 : ascorbic acid 1; 21°C. (*1*) Experimental curve and (*2*) theoretical dependence.

Presumably, under the conditions of radical degradation in the presence of redox system, the degradation reactions yielding low-molecular-weight chitosan compete with other reactions involving free radicals. In particular, radicals can decay in the reactions with ascorbic acid [9]. In this case, an increase in the ascorbic acid concentration at a constant substrate concentration may increase the relative rate of reactions leading to the decay of radicals. Furthermore, low-molecular-weight chains also participate in the decay of radicals. We suggest the following reaction scheme.

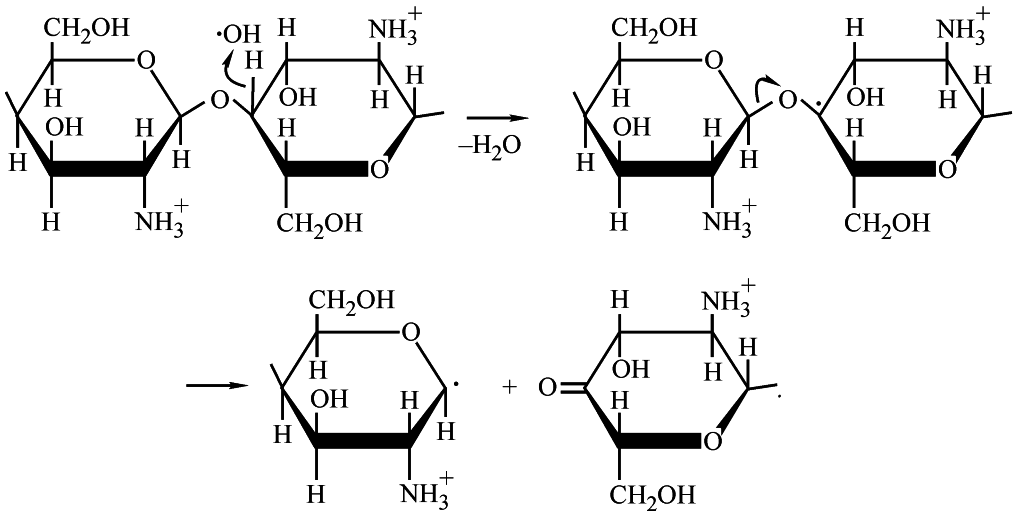
Generation of radicals by the reaction of hydrogen

peroxide with ascorbic acid (AAH_2 is ascorbic acid and AA, dehydroascorbic acid):

$$AAH_2 \rightleftarrows AAH^{\bullet} + H_2O + OH^{\bullet},$$

$$AAH^{\bullet} + H_2O_2 \rightarrow AA + H_2O + OH^{\bullet}.$$

Cleavage of the chitosan backbone with the formation of low-molecular-weight products:



Deactivation of radicals:

$$AA^{-} + OH^{\bullet} \rightarrow AA + OH^{-},$$

$$AAH^{\bullet} + OH^{\bullet} \rightarrow AA + H_2O,$$

$$OH^{\bullet} + AAH^{-} \rightarrow AAH^{\bullet} + OH^{-},$$

$$OH^{\bullet} + AAH_2 \rightarrow AAH^{\bullet} + H_2O,$$

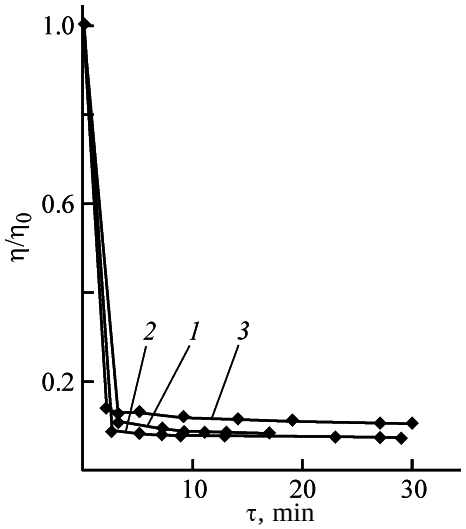
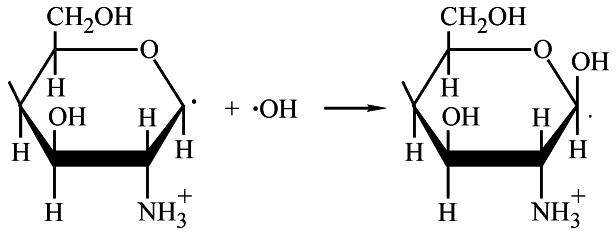


Fig. 5. Variation of the viscosity η/η_0 of chitosan solutions with time τ at (*1*) 24, (*2*) 40, and (*3*) 60°C. Concentrations in solution, wt %: chitosan 2 and acetic acid 1.5; molar ratios: chitosan : H_2O_2 40 and H_2O_2 : ascorbic acid 2.



The rate of the latter reaction should sharply increase as the low-molecular-weight chitosan is accumulated in the reaction mixture with an increase in the concentration of the initiating system.

The influence of temperature on the degradation of chitosan chains was examined by comparing the initial reaction rates and molecular weights of products in the range 0–60°C. We found that raising the temperature only slightly affects the rate at which the viscosity of chitosan solutions decreases (Fig. 5). Such a behavior is consistent with the general concept that processes occurring by the redox mechanism have a low activation energy. The activation energy calculated from data in Fig. 5 is 5 kJ.

The molecular weight of chitosan after the degradation at 0°C was 8.5×10^3, against 9.5×10^3 at 21°C and equal other conditions.

CONCLUSIONS

(1) Chitosan with a degree of polymerization of 10–40 can be prepared by low-temperature chemical

degradation of high-molecular-weight natural chitosan using the hydrogen peroxide–ascorbic acid redox system.

(2) It is the most appropriate to perform the process at molar ratios H_2O_2 : substrate 0.1 and H_2O_2 : ascorbic acid 1 : 1.

(3) The chitosan degradation rate weakly depends on temperature (activation energy 5 kJ).

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