Chemiluminescence accompanying chitosane destruction in hydrochloric acid solution containing hydrogen peroxide

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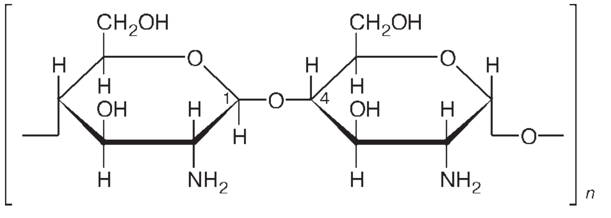
The chemiluminescence (CL) accompanies the destruction of chitosane (CTS) in the presence of H_2O_2. The CL kinetics has a complex character and can be described as a sum of two exponentials. The photoluminescence monitoring and IR spectra of the reaction mixture indicate the formation of carbonyl groups in the course of CTS destruction. The enhancement of the CL intensity by Fe^{2+} suggests the reaction of Fe^{2+} with hydroperoxides accumulated with CTS decomposition. The macroradicals $ROO^{\bullet}$ are assumed to be transformed through the cleavage of the glycoside bond *via* a six-membered cyclic intermediate to form the terminal peroxide radical and ketone.

Key words: chitosane, chemiluminescence, destruction, hydrogen peroxide.

Synthesis of water-soluble oligosaccharides of chitosane (CTS) is a necessary stage in manufacturing various drugs.[1] Several methods for preparing water-soluble oligomers, including oxidative destruction and hydrolysis in acidic and alkaline solutions, are known.[2,3] A series of requirements are imposed on the chemical methods for chitosane destruction, the main of which being simplicity of removal of reactants and high destruction rate.

The main method for studying oxidative destruction is measurement of viscosity, which depends on the mean molecular weight of oligosaccharides that formed. At the same time, a search for other methods for studying oxidative destruction of polysaccharides along with traditional methods will make it possible, in our opinion, to reveal some details of the mechanism of their decomposition.

In this work, we studied chitosane destruction in the presence of H_2O_2 in hydrochloric acid solution using chemiluminescence (CL), *viz.*, a phenomenon, which is directly related to the reaction rate.



CTS

Experimental

Chemiluminescence of a hydrogen chloride solution of CTS in the presence of H_2O_2 was monitored on a photometric installation using a FEU-140 photomultiplier. Photoluminescence spectra (PL) were obtained on a Hitachi MPF-4 spectrofluorimeter. IR spectra were recorded on a Specord M80 spectrophotometer. Experiments were carried out at 30 °C. Intrinsic viscosities were measured on Ubellohde viscosimeters.

Chitosane from Far-Eastern crabs (poly[(1→4)-2-amino-2-deoxy-β-D-glycose], RIG Ltd. (Russia), Technical Standard 6-09-05-397-75), was dissolved in 1 *N* HCl and precipitated by 2 *N* NaOH. The precipitate was washed by distilled water and dried. Hydrogen peroxide (special-purity grade) and other reagents were used without additional purification.

Results and Discussion

Chitosane destruction in a hydrochloric acid solution containing no H_2O_2 is due to the proton attack at the 1,4-glycoside-glycose bonds.[2] It has been established[4] that in the presence of H_2O_2 the rate of CTS destruction increases substantially likely due to a free-radical destruction mechanism. Therefore, in the presence of O_2, the appearance of chemiluminescence is expected.[5] Indeed, the interaction of a hydrochloric acid solution of chitosane with H_2O_2 in air is accompanied by CL. In an inert N_2 atmosphere, the CL intensity decreases to the level deter-

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 1, pp. 52—55, January, 2002.

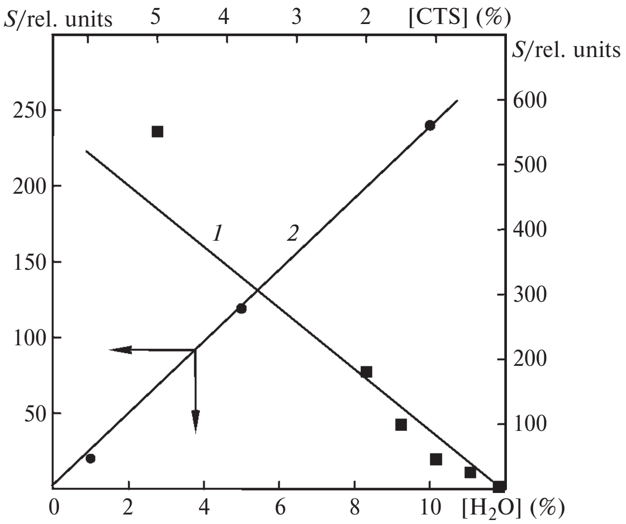


Fig. 1. Total radiation of CL (S) as a function of concentrations of H_2O_2 ([CTS] = 1%) (*1*) and CTS ($[H_2O_2]$ = 2%) (*2*).

mined by the dark current of a photoelectron multiplier. A change of the inert atmosphere for air recovers CL.

Due to a complex structure of CTS solutions and their high viscosity, the intensity of the observed CL is poorly reproduced (20—25% between parallel experiments) and, hence, we used the total radiation as the integral measure of the reaction. The total CL radiation increases linearly with an increase in concentrations of both H_2O_2 and CTS (Fig. 1), which indicates the origination of CL from the interaction of the reactants or intermediates formed from them.

Figure 2, *a* presents a typical curve for CL decay (*1*) during CTS destruction. The curve has a complicated shape and can be satisfactorily described by the sum of two exponentials

$$I_{CL} = a_1\exp(-k_1t) + a_2\exp(-k_2t),$$

where k_1 and k_2 are the apparent rate constants for CL decay. The k_1 and k_2 constants were obtained by processing the CL decay curves using the non-linear regression method (Fig. 3). The obtained k_1 and k_2 constants are, in our opinion, the limiting values, which restrict the interval of the constants variation, *i.e.*, their dispersion. It is more correct to represent the experimental kinetics of CL decay as the sum of the greater number of exponentials, which reflect the interaction of the destructing reactant with CTS. As can be seen in Fig. 3, *b*, the dispersion of k_1 and k_2 increases with an increase in the CTS concentration.

The complicated shape of the CL decay curve and the increase in dispersion of the constants are due, most likely, to specific features of the structure of the polymeric CTS molecule. Polysaccharides, being flexible macromolecules, form a ball and diffuse rather slowly as compared to solvent molecules. Therefore, reactions of the polymer ball with small molecules are mainly determined by diffusion of low-molecular reactants, and the position of the center of gravity of the macromolecule can be considered unchanged in the course of reaction. The macromol-

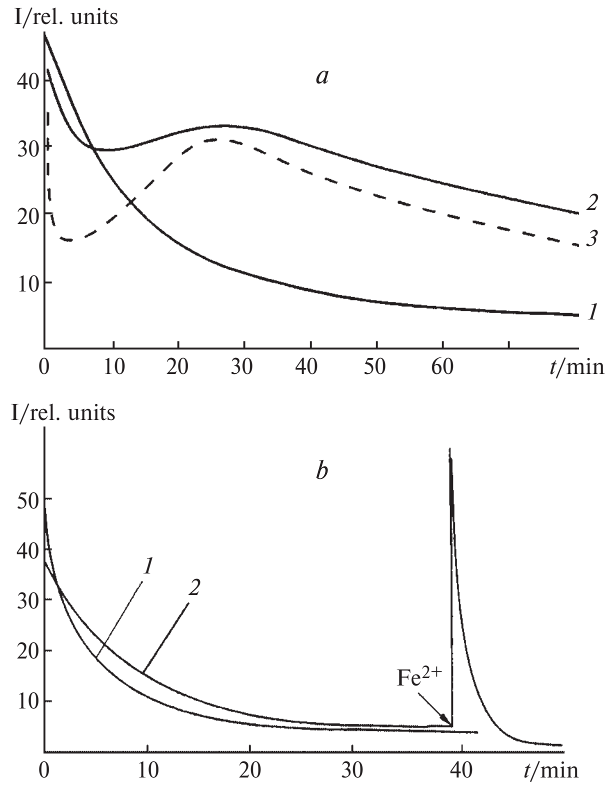


Fig. 2. CL kinetic curves for oxidative destruction of CTS ([CTS] = 1%, $[H_2O_2]$ = 2%). *a*: *1*, 30; *2*, 60; *3*, 80 °C. *b*: upon addition of Fe^{2+} (10^{-4} mol L^{-1}) at the initial moment of destruction (*1*) and at the moment of reaching a constant CL intensity (*2*) at 30 °C.

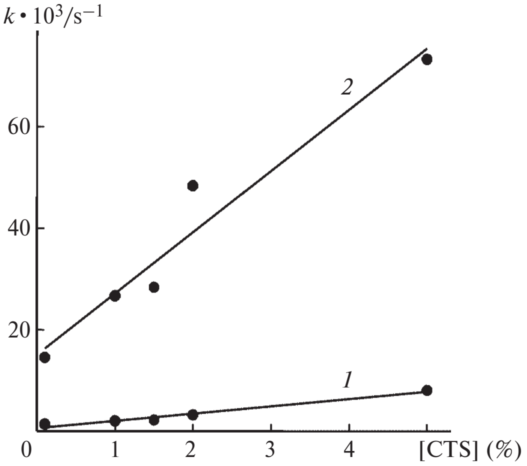


Fig. 3. Influence of the CTS concentration on the minimum (*1*) and maximum (*2*) rate constants of CL decay at $[H_2O_2]$ = 2%.

ecule—reactant space distribution is always random due to their high mutual mobility, and the probability of the reaction is independent of the localization of the reactant in the medium, *i.e.*, the reactant interacts with the polymer over the whole ball bulk. However, high dispersion of the apparent rate constants is determined, probably, by the steric factors of the reaction of intermediate species. When a solution of polymer is diluted, the random distribution is violated, individual molecular balls are formed, and groups with close reactivities, which are only on the macromolecule surface, become accessible for the reaction.

The data on the CL decay kinetics at different temperatures (see Fig. 2, *a*) show that at 60 and 80 °C the glow curves pass through a maximum, indicating the formation and consumption of an intermediate compound. Photoluminescence monitoring of the reaction mixture during CTS destruction indicates the accumulation of chromophoric molecular groups, which emit in a region of 400—450 nm. The increase in the intensity of the band at 1720 cm^{-1} in the IR spectrum of the reaction mixture indicates unambiguously the formation of the carbonyl groups during destruction. We suggest that the carbonyl groups, which are the CL emitters, are also formed at moderate temperatures (~30 °C) because the CL spectrum coincides with that of photoluminescence of CTS subjected to destruction (Fig. 4).

The addition of Fe^{2+} sulfate (10^{-4} mol L^{-1}) enhances CL and increases the rate of its decay. However, this effect is dissimilar at different stages of CTS destruction. The addition of Fe^{2+} at the initial moment of the reaction only accelerates CL decay, whereas a glow flash is observed when Fe^{2+} is introduced at the moment when the CL reaches an almost constant level of intensity (see Fig. 2, *b*). The influence of the Fe^{2+} ions on CL at the initial moment of destruction is a result of their catalytic effect on the decomposition of H_2O_2 according to the Haber—Weiss scheme[6] and the reaction of the formed active oxygen-containing radicals with the polymer. The interaction of these species with organic substrates results, as a rule, in the formation of peroxides. It is likely that hydroperoxides are also accumulated during the oxidation of the polymer with H_2O_2, and their reaction with the Fe^{2+} ions results in the CL flash when the glow reaches a constant level. Peroxide formation can additionally be confirmed as follows. Thermolysis at 30 °C of a chitosane suspension subjected to destruction with a solution of H_2O_2 and then washed from the latter is accompanied by a glow, whose spectral characteristics and decay kinetics are similar to those of CL under homogeneous conditions.

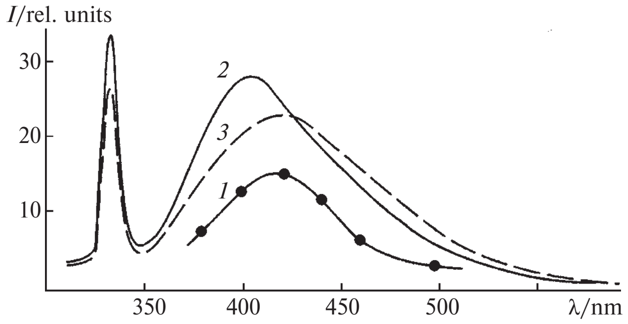


Fig. 4. Chemiluminescence spectrum (*1*) and photoluminescence spectra of the starting (*2*) and subjected to destruction CTS (*3*) under irradiation with λ = 310 nm.

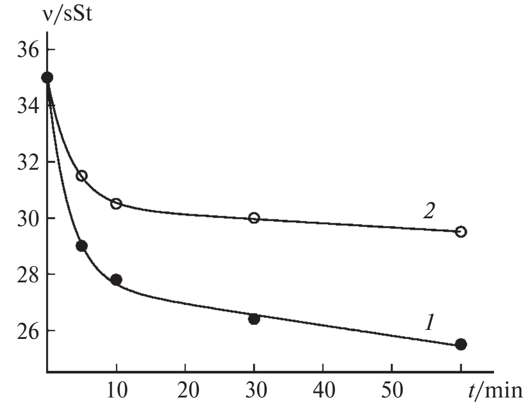
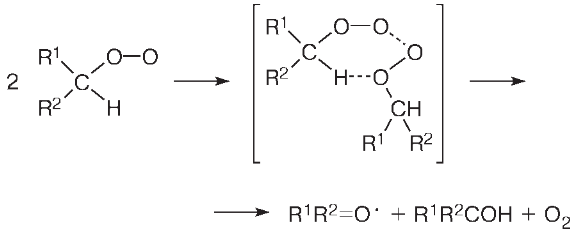


Fig. 5. Kinetic curves for destruction of a 1% solution of CTS in 0.1 *N* HCl in the presence of 2% H_2O_2 (*1*) and 0.08% Trilon B (*2*) at 30 °C.

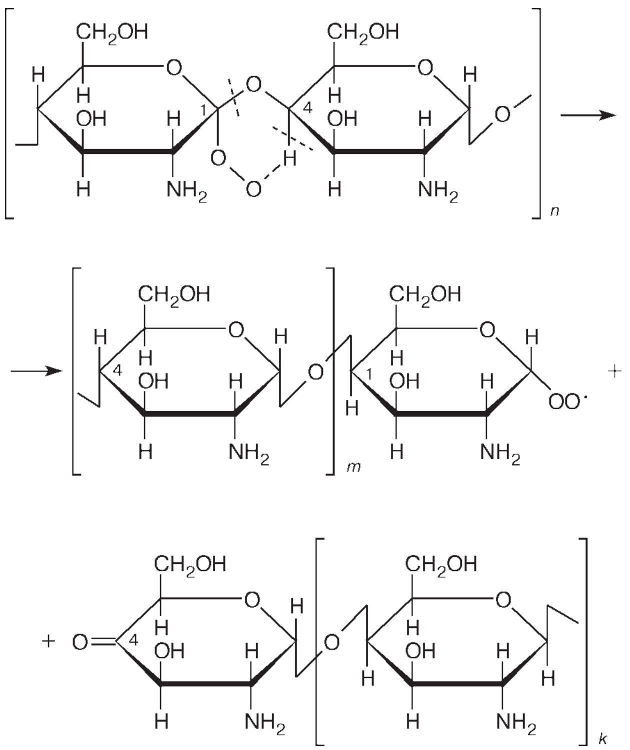
Probably, the observed CL and, correspondingly, CTS destruction are due to reactions of radical species formed during H_2O_2 decomposition catalyzed by the traces of transition metal ions, which are present in the reaction mixture. This is confirmed by the influence of sodium ethylenediaminetetraacetate (Na_2EDTA) on CL. In the presence of Na_2EDTA, the CL intensity is much lower than that of the reference solution. The CL kinetics also changes: the glow decays more slowly. It is known that Na_2EDTA forms stable complexes with transition metals and, most likely, these complexes catalyze H_2O_2 decomposition more weakly.

Comparison of the CL kinetics and kinetics of changing the viscosity of a CTS solution proves that CL reflects CTS destruction (see Figs. 2 and 5).

Scheme 1



Scheme 2



The most general mechanism of CL appearance in oxidation of organic compounds is the disproportionation of the secondary peroxide radicals[7] (Scheme 1).

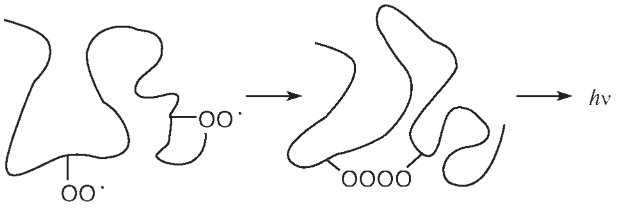
Taking into account these facts and the above-mentioned data, we can assume that the observed CL is related to the disproportionation of the $RO_2^{\bullet}$ radicals formed in the polymer chain. CTS destruction is likely due to the $OH^{\bullet}$ radicals formed in the catalytic decomposition of H_2O_2 because the H atoms in the C(1) and C(4) positions are most weakly bound and, probably, they are precisely the H atoms which interact with the $OH^{\bullet}$ radicals. In the general form, the reactions occurred in the course of CTS destruction can be presented as follows.

$$Me^{n+} + H_2O_2 \longrightarrow Me(OH)^{n+} + OH^{\bullet},$$

$$RH + OH^{\bullet} \longrightarrow R^{\bullet} + H_2O,$$

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet} \text{ (in polymer chain).}$$

The macroperoxide radicals that formed disproportionate through the intermediate tetraoxide, which, according to Scheme 1, decomposes with light emission.



In turn, the $ROO^{\bullet}$ macroradicals are transformed with the glycoside bond cleavage. This transformation (Scheme 2) with the formation of the terminal peroxide radical and ketone occurs, probably, through an intermediate product.

Thus, we showed that CTS destruction in hydrochloric acid solutions in the presence of H_2O_2 proceeds through the formation of the $ROO^{\bullet}$ radicals, whose disproportionation results in CL.

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Received February 18, 2000;
in revised form August 6, 2001