OH-Radical-induced chain scission of chitosan in the absence and presence of dioxygen

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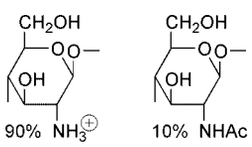
Hydroxyl radicals (and 15% H-atoms) were generated radiolytically in N_2O-containing aqueous solutions of protonated chitosan ($M_w = 4.0 \times 10^5$ Da, degree of deacetylation 90.5%, pH ~ 3). The rate constant of H-abstraction from chitosan by OH radicals is $k = 6.4 \times 10^8$ dm^3 mol^{-1} s^{-1}, as measured by pulse radiolysis using thymine as competitor. With SCN^- as competitor the apparent rate constant is found to be too high, because of a condensation of SCN^- around the positively charged macromolecule. The radiation-chemical yields of chain scission are $G = 3.4 \times 10^{-7}$ mol J^{-1} in N_2O-saturated and $G = 2.1 \times 10^{-7}$ mol J^{-1} in N_2O–O_2-saturated solutions. The kinetics have been followed by pulse radiolysis with conductometric detection. For each chain break, ~3.1 counterions (ClO_4^-) are released, on average, from the condensation zone into the bulk solution. In N_2O-saturated solution the kinetics of chain scission are independent of dose rate, although more than one first-order process contributes (overall: $k \approx 95$ s^{-1} at pH 3.3). In the presence of dioxygen, the kinetics of chain scission depend on the dose rate, *i.e.* second-order processes play also a significant role.

Chitosan, a copolymer of β-(1→4)-D-glucosamine and *N*-acetyl-β-(1→4)-D-glucosamine, is a water-soluble cationic polysaccharide of natural origin. Although chitosan itself is found in nature, for practical purposes it is usually obtained by deacetylation of the much more abundant chitin. Basic data on the synthesis, properties and applications of chitosan can be found in refs. 1–3.



Although the range of applications of this polymer is surprisingly broad (*e.g.* in mining, textile manufacturing, wastewater purification and cosmetics), it owes much of the world-wide interest in it to its valuable biomedical properties.[4,5] For example, the acceleration of wound healing, a hypocholesterolemic activity and even the suppression of some tumors have been reported.[1,2,6–9]

There are a number of reasons why the study of the free-radical-induced reactions of chitosan under the action of ionizing radiation is considered to be of some importance. Biomaterials containing chitosan have to be sterilized, and radiation sterilization is often the method of choice.[10] Ionizing radiation is also an increasingly popular tool for the formation of bio-compatible materials, especially hydrogels.[11–13] The suitability of chitosan for a particular biomedical application depends on its molecular weight,[4,10,14–17] and the reduction of the molecular weight by irradiation is a simple, labour-saving and environment-friendly alternative to the classical acid hydrolysis. Efficient and safe application of radiation techniques in the processing of chitosan and chitosan-containing products requires the underlying free-radical chemistry to be known in some detail.

Most of the previous studies on the radiolysis of chitosan were done with solid samples.[10,18–24] Although irradiation of dry chitosan is a very simple way to reduce its molecular weight, this also has drawbacks, namely long-lasting post-irradiation effects (further decrease in molecular weight due to slow reaction of the radicals trapped in the solid) and the occurrence of side reactions.[10,21–23] Therefore, irradiation in aqueous solution (or in a water-swollen state), where no post-irradiation effects are observed and also the extent of side reactions is apparently smaller, seems to be an alternative, at least as a method for molecular weight reduction. Limited data on irradiation in solution are available,[19,21,25,26] and mechanistic details are largely unknown.

In a preliminary study, co-authored by one of us, an attempt was made to measure the radiation-induced chain scission of chitosan in aqueous solution by pulse radiolysis with right-angle laser light-scattering.[21] The necessity of using very high doses per pulse and the sensitivity of the right-angle light-scattering signal to conformational changes prevented a clear-cut interpretation of those data. In particular, the scission yields have been much underestimated.

In the present work, we used the pulse radiolysis technique with both optical and conductometric detection (particularly suitable for low-dose experiments on scission reactions of polyelectrolytes) to study the kinetics of the most important OH-radical-induced reactions of chitosan in deoxygenated and oxygenated aqueous solutions, as well as low-angle laser light-scattering as an absolute method for determination of the changes in molecular weight upon irradiation of chitosan solutions with γ-rays.

Experimental

Chitosan was prepared by deacetylation of krill (*Euphausia superba*) chitin with 50% aqueous sodium hydroxide at a chitin weight fraction of 10% at 100 °C for 20 min, followed by

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washing with water. This procedure was carried out three times. The degree of deacetylation was 90.5% as determined by potentiometric titration,[27] and the weight-average molecular weight was $M_w = 4.0 \times 10^5$ Da, as measured by the low-angle laser light-scattering (see below). According to dialysis tests (Amicon TCF10 with a Diaflo YM10 membrane), the molar fraction of low-molecular-weight material ($M < 10^4$ Da) was lower than 10%. Polymer concentrations are given in mol dm^{-3} of the repeating unit (on average 165 Da).

Perchloric acid was chosen to dissolve chitosan, since perchlorate does not react with the reactive products of water radiolysis. Chitosan powder was dispersed in Milli-Q-filtered water (Millipore) with constant stirring and stoichiometric amounts of 1 mol dm^{-3} $HClO_4$ were added. Stirring was continued for 2 h to ensure dissolution was complete. Subsequently, the solution was passed through a filter (5 μm pore size, Minisart, Sartorius). Storage of the solution for up to 48 h did not lead to any noticeable decrease in molecular weight. If necessary, the pH was adjusted by further $HClO_4$ addition directly before the experiment. Prior to irradiation, solutions were saturated for 1 h with N_2O, purified by an Oxisorb column (Messer-Griesheim), or with an N_2O–O_2 (4 : 1 v:v) gas mixture.

γ-Irradiations were carried out with a panoramic ^{60}Co-γ-source (Nuclear Engineering) at dose rates of 0.082 Gy s^{-1} or 2.9 Gy s^{-1}. For pulse radiolysis measurements, a Van-de-Graaff accelerator generating 0.4 μs pulses of 2.8 MeV electrons was used, equipped with optical and conductometric detection systems.[28,29] Fricke dosimetry was used for γ-irradiations,[30] while for optical and conductometric pulse experiments thiocyanate and dimethyl sulfoxide dosimetries[31–33] were applied. In conductometry, dimethyl sulfoxide dosimetry allows us to determine yields accurately, but the dose remains unknown. In principle, this can also be determined accurately,[34] but this method is laborious and has not be done here, since the dose can be estimated to an accuracy of ±25% from the positioning of the cell and dosimetry by optical detection. For the purpose of the information to be drawn from Fig. 5, this accuracy was quite sufficient.

Weight-average molecular weights were determined by low-angle laser light-scattering (Chromatix KMX-6 equipped with a He/Ne laser, $\lambda = 633$ nm, scattering angle 6–7°) at pH 3 ($HClO_4$) in solutions containing 0.25 mol dm^{-3} $NaClO_4$ in order to maintain the coiled conformation of polymer chains. Directly before the light-scattering measurements, solutions were passed through a filter (0.45 μm pore size, Millex-HA, Millipore). The refractive index increment for chitosan in this solvent was determined with a laser differential refractometer (Chromatix KMX-16, $\lambda = 633$ nm) at 25 °C to be $dn/dc = 0.187$ cm^3 g^{-1}.

Results and discussion

The free-radical generating system

When dilute aqueous solutions of chitosan are subjected to ionizing radiation, most of the radiation energy is absorbed by water, while the absorption of ionizing radiation by the polymer itself can be neglected. Thus, the free-radical reactions are induced by the radicals formed upon the absorption of the ionizing radiation by water [*cf.* reaction (1)].[35] The radiolysis of water yields OH radicals, hydrated electrons and hydrogen atoms. Their radiation-chemical yields, expressed as G values (unit: 10^{-7} mol J^{-1}) [$G(^{\bullet}OH) \approx G(e_{aq}^-) = 2.8 \times 10^{-7}$ mol J^{-1}, $G(H^{\bullet}) = 0.6 \times 10^{-7}$ mol J^{-1}] are identical for both kinds of ionizing radiation used in this study, *i.e.* ^{60}Co-γ-rays and high-energy electrons.

The hydrated electron can be readily converted into further $^{\bullet}OH$ by saturating the solution with N_2O [reaction (2), $k = 9.1 \times 10^9$ dm^3 mol^{-1} s^{-1}].[36] In the acidic pH range used in this study, this conversion is not quantitative because of competition by the proton [reaction (3), $k = 2.3 \times 10^{10}$ dm^3 mol^{-1} s^{-1}].[36] Nevertheless, even at the lowest pH used (pH 3.0), the N_2O-containing system consists of 85% $^{\bullet}OH$ and only 15% $H^{\bullet}$. Since H atoms also undergo H-abstraction (see below), albeit at a lower rate, we can add them to the OH-radical yield without inducing a major error.

$$H_2O \xrightarrow[\text{radiation}]{\text{ionizing}} e_{aq}^-,\ ^{\bullet}OH,\ H^{\bullet},\ H_2O_2,\ H_2,\ H^+,\ OH^- \quad (1)$$

$$e_{aq}^- + N_2O \longrightarrow {}^{\bullet}OH + N_2 + OH^- \quad (2)$$

$$e_{aq}^- + H^+ \longrightarrow H^{\bullet} \quad (3)$$

The reactivity of hydrated electrons towards carbohydrates is too low (typically $k < 5 \times 10^6$ dm^3 mol^{-1} s^{-1})[35–37] to compete with reactions (2) and (3) under our experimental conditions.

Reaction of OH radicals with chitosan

Hydroxyl radicals react with low-molecular-weight carbohydrates by abstraction of carbon-bound hydrogens with a rate constant $k > 1 \times 10^9$ dm^3 mol^{-1} s^{-1}, while the reactivity of H atoms is more than an order of magnitude lower.[36,38] Due to a different reaction geometry, the rate constants of the reactions of OH radicals with polymers, when expressed in dm^3 (mol of repeating unit)$^{-1}$ s^{-1}, are lower than for the low-molecular-weight analogues. They depend on the molecular weight and conformation of the macromolecules and, to a certain extent, also on their concentration.[39–42]

Very few data on the rate constants of H-abstraction from polysaccharides by OH radicals are available. For hyaluronic acid of an average molecular weight $M > 10^6$ Da, $k = 7$–9×10^8 dm^3 mol^{-1} s^{-1} has been reported,[37,43] while for dextran of $M \approx 10^5$ Da, a value in the order of only 1×10^7 dm^3 mol^{-1} s^{-1} is calculated from the data of ref. 39. This difference may be, at least in part, attributed to the various conformations of these polymers (an open conformation close to a rigid rod in the charged hyaluronic acid *vs.* coiled conformation of dextran). Also for synthetic weak polyelectrolytes the rate constants are higher when the macromolecules are charged and attain a linear conformation.[41,44,45]

Although, in principle, a direct pulse-radiolytic measurement of the rate constant of $^{\bullet}OH$ with a substrate can be made by following the build-up of the absorbance of a product after an electron pulse, in most cases measurements based on competition kinetics are more suitable.[35,36] The latter method was also chosen in the present case, since chitosan-derived radicals show only a weak absorption in the accessible wavelength region ($\varepsilon \sim 300$ dm^3 mol^{-1} cm^{-1} at around 300 nm, *cf.* Fig. 2 and ref. 21).

If the absorption of the product resulting from the reaction of $^{\bullet}OH$ with the competing scavenger is denoted as A_0 in the absence of chitosan and as A in its presence, while [chitosan] and [scavenger] are the molar concentrations of the respective substances, relation (4) holds.[30,35]

$$\frac{A_0}{A} - 1 = \frac{k(^{\bullet}OH + \text{chitosan}) \times [\text{chitosan}]}{k(^{\bullet}OH + \text{scavenger}) \times [\text{scavenger}]} \quad (4)$$

To assure that the necessary assumption of a statistical distribution of the competitor in the reaction volume is fulfilled in the case of polyelectrolytes, a neutral species (or a species having a charge of the same sign as the polyion) should be chosen. We have used thymine, which upon OH attack forms adducts absorbing around $\lambda = 380$ nm[46] ($k = 6.4 \times 10^9$ dm^3 mol^{-1} s^{-1}; value recommended for competition studies[36]). From the dependence of the absorption at 380 nm, observed after an electron pulse, on the molar ratio chitosan/thymine (filled circles in Fig. 1), the rate constant of $^{\bullet}OH$ with chitosan is

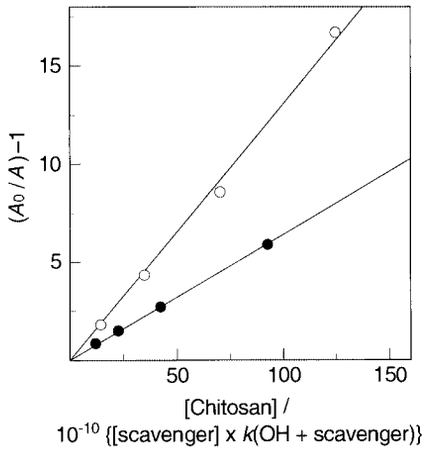


Fig. 1 Pulse radiolysis of chitosan (1×10^{-2} mol dm^{-3}, pH 3.0) in N_2O-saturated aqueous solution. Determination of the rate constant of reaction (4) by the competition method. Absorption ratio of competitor transient in the absence and presence of chitosan (A_0/A) as a function of the ratio of chitosan concentration to the scavenging capacity of the competitor [see equation (4)]. Competitors: thymine (●) and SCN^- (○). Dose per pulse, 1.7 Gy.

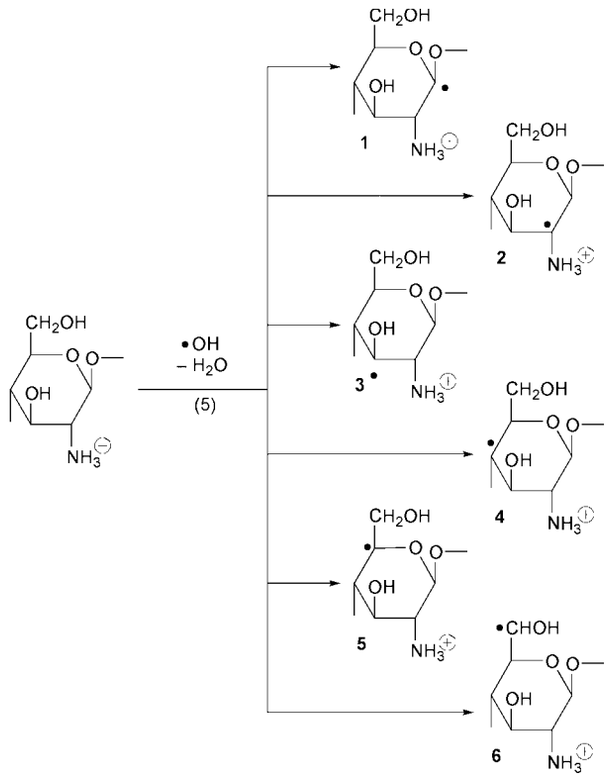
calculated as $k = 6.4 \times 10^8$ dm^3 mol^{-1} s^{-1}, *i.e.* similar to the values reported for hyaluronic acid. In a strict sense, this value is valid only for our chitosan sample; rate constants for samples of different molecular weight distribution and/or degree of deacetylation may differ noticeably.

In order to illustrate the effect of a competitor having a charge opposite to that of the polymer, measurements were also carried out with SCN^-. These anions are expected to accumulate in the layer of condensed counterions surrounding the positively charged (protonated) chitosan chains. When the overall SCN^- concentration is, as in our experiment, much lower than the concentration of the charged monomer units, this effect should lead to a significant depletion of SCN^- in the bulk solution, relative to the immediate vicinity of chitosan. The total SCN^- concentration on which the competition calculations are based is therefore higher than its concentration in the bulk solution, and this leads to an overestimation of the rate constant of $^{\bullet}OH$ with chitosan. The apparent value of 1.3×10^9 dm^3 mol^{-1} s^{-1} (Fig. 1, open circles) is twice as high as the value obtained with the neutral scavenger. This indicates a drop in the bulk SCN^- concentration by approximately the same factor.

Product analysis and EPR data for simple, unsubstituted mono- and polysaccharides show that the selectivity of the reaction of $^{\bullet}OH$ with these species is low,[35,47,48] *i.e.* carbon-bound hydrogen atoms are abstracted from all positions with a similar probability [reaction (5)].

In the present case, the influence of substituents must be taken into account. The acetamido group at C(2) does not seem to have much influence on the selectivity, and there is no evidence for a major $^{\bullet}OH$ attack on this group.[49] On the other hand, the protonated amino group at C(2) is expected to have a more pronounced effect on the selectivity of H-abstraction by electrophilic hydroxyl radicals, as has been shown for 2-amino-2-deoxy-D-glucose.[35,50] Since upon protonation of the amino group electron density is withdrawn from C(2), this position is less likely to be attacked. Other positions must profit from this effect, and an increased attack at C(1) and C(4), leading to the formation of a carbon-centered radical directly at the glycosidic linkage, should result in a relatively high yield of chain scission, which is indeed observed (see below).

The absorption spectrum of chitosan-derived radicals (Fig. 2) is not very characteristic and shows only an increasing absorption towards shorter wavelengths, but its change with time yields some information (see below).



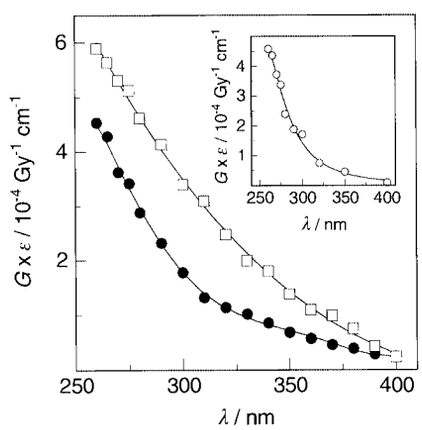


Fig. 2 Pulse radiolysis of chitosan (1×10^{-2} mol dm^{-3}) in N_2O-saturated aqueous solution, pH 3.0. Absorption spectra of polymer radicals taken at 50 μs (●) and 5.0 ms (□) after the electron pulse, 3.4 Gy. Inset: spectra recorded in N_2O–O_2 saturated solutions, pH 3.0, 10 μs after the pulse.

Chain scission in deoxygenated solutions

One of the most important radical-induced reactions of oligo- and polysaccharides is the scission of the glycosidic bond. Thus, lower oligomers or monosaccharides are formed upon irradiation of oligosaccharides, while irradiation of polysaccharides results in a decrease of the average molecular weight. The scission yield somewhat depends on the type of glycosidic bond.[51] For cellobiose and lactose, having β-1→4 linkages as in the case of chitosan, the radiation-chemical yields of scission in dioxygen-free, N_2O-saturated solutions were measured as $G_s = 2.5 \times 10^{-7}$ mol J^{-1} and $G_s = 2.2 \times 10^{-7}$ mol J^{-1}, respectively,[51] *i.e.* 35–40% of the initially formed radicals lead to the scission of the glycosidic bond. This is in agreement with the observation that only the radicals next to the glycosidic bond [C(1) and C(4)], and also probably that at C(5), undergo scission.[50]

The decrease in molecular weight of chitosan upon γ-irradiation is shown in Fig. 3 (filled circles). Assuming that no

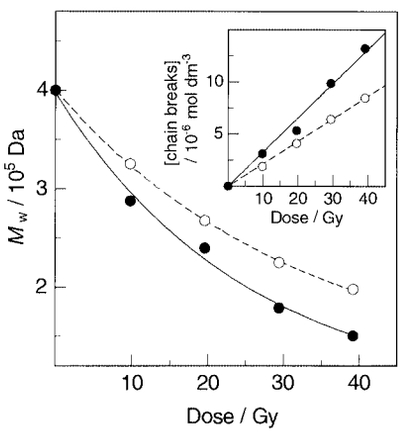


Fig. 3 γ-Radiolysis of chitosan (1×10^{-2} mol dm^{-3}, pH 3.0) in N_2O-saturated (●) and N_2O–O_2-saturated (○) aqueous solution. Dose rate, 0.082 Gy s^{-1}. Weight-average molecular weight as a function of dose. Inset: concentration of chain breaks as a function of dose.

crosslinking takes place (see below), these data were converted into the concentration of chain breaks (inset in Fig. 3, filled circles) according to eqn. (6),[52,53] where M_{w0} and M_w are the

$$[\text{Chain breaks}] = 2 \times (M_w^{-1} - M_{w0}^{-1}) \times c \qquad (6)$$

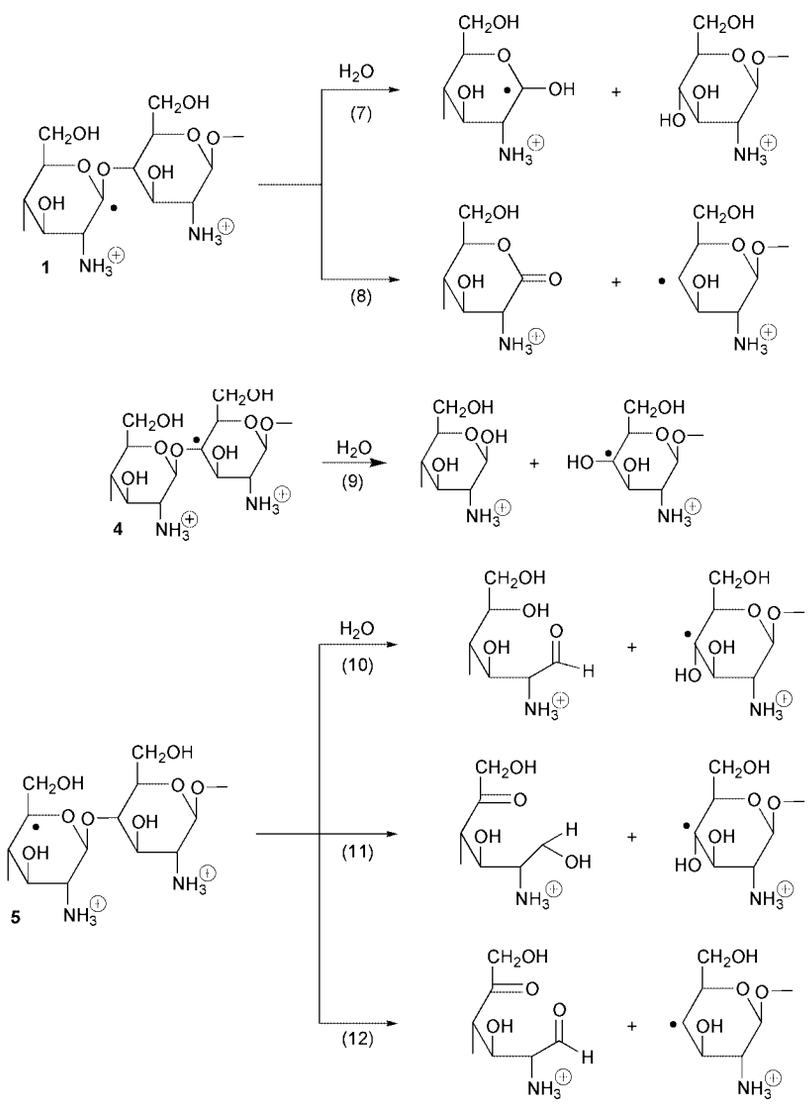
weight-average molecular weights (in g mol^{-1}) before and after irradiation, with c being the concentration of polymer in g dm^{-3}.

From the dose-dependence of the chain break concentration, the radiation-chemical yield of chain scission is calculated at $G_s = 3.4 \times 10^{-7}$ mol J^{-1}. This value is somewhat higher than expected for the yield of precursor radicals C(1), C(4) and C(5) if the OH-attack is fully random, and is also higher than that of cellobiose and lactose. This result is in accordance with the above-mentioned expectation that the other positions profit from a reduced attack at C(2) due to the neighbouring protonated amino group.

The possible reaction pathways of chain breakage, although not investigated in the present study, are expected to follow the general scheme of the scission of glycosidic bonds studied in detail for disaccharides.[35,50,51,54] The main mechanisms are hydrolysis [reactions (7), (9), (10)] and fragmentation [reactions (8), (11), (12)] of radicals at C(1), C(4) and C(5).

In the case of disaccharides, it has been suggested that the scission of glycosidic bond is relatively fast ($k \geq 35$ s^{-1}).[35] In fact, upon pulse-irradiation of N_2O-saturated chitosan solutions, an increase in absorbance is observed in the time-scale of a few milliseconds (compare the spectra at 50 μs and 5 ms in Fig. 2). However, this optical change cannot be attributed with certainty to the chain scission, since other reactions, such as water eliminations, occur as well,[50] and the absorption spectra of the resulting radicals are also characterised by a somewhat stronger absorption.

For charged polysaccharides, however, pulse radiolysis with conductometric detection (*cf.* refs. 28,29) provides a better insight into the kinetics of glycosidic bond scission. The application of this method for following the chain scission in polyelectrolytes is based on the fact that the high linear charge



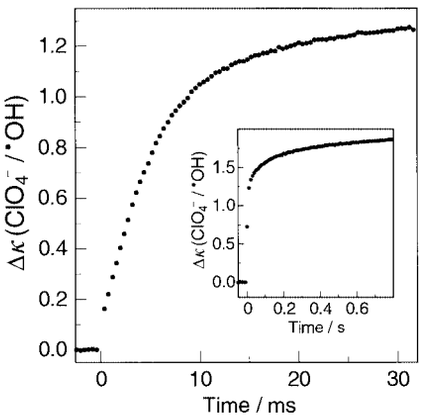


Fig. 4 Pulse radiolysis of chitosan (5×10^{-4} mol dm^{-3}) in N_2O-saturated aqueous solution, pH 3.3, 9 Gy. Conductivity increase (mol ClO_4^- released per mol $^{\bullet}OH$ reacted) as a function of time after pulse. Inset: Expanded time range.

density of a polyion causes most of the counterions to condense around the chain. Condensed counterions, although not bound to specific ionic groups of the polyion, can only move along the potential valley of the polyelectrolyte chain, and cannot escape from its electrostatic field.[55–57] Therefore, these counterions do not contribute to the conductivity. When chain scission occurs and the broken ends diffuse apart, the electrostatic potential around the newly formed chain ends is lowered, and a few counterions are released into the bulk solution. This leads to an increase in conductivity.[58] This method has been successfully applied in many studies on chain scission in natural and synthetic anionic polyelectrolytes.[43,58–63]

Since in our experiments chitosan was dissolved in water by addition of perchloric acid, the layer of condensed counterions is composed of perchlorate anions. When N_2O-saturated chitosan solution is subjected to a pulse of high-energy electrons, a pronounced increase in conductivity is observed (Fig. 4).

The overall half-life of this process does not depend on the dose per pulse. This indicates that, as expected, scission is a unimolecular reaction initiated by transformations of radicals. However, the conductivity increase cannot be satisfactorily described by simple first-order kinetics [*cf.* the slower process(es) shown in the inset of Fig. 4]. This is most probably caused by the different reactivities of the scission-initiating radicals. The overall rate constant of chain scission, based on the conductivity data, is $k \approx 95$ s^{-1}. This value is in agreement with that ($k \geq 35$ s^{-1}) given for the analogous reactions of disaccharides.

Measurements on another ionic polysaccharide, hyaluronic acid, revealed that the rate constant of chain scission depends on pH, *i.e.* can be both base- and acid-catalysed, as expected for the hydrolysis reactions involved.[43] Thus, also for chitosan, one can expect a pH-dependence of the scission rate constant. This dependence is, however, not easily measured with the conductometric technique, since the pH window for these measurements is limited on one side to pH < 3.8 by the solubility of chitosan and on the other side to pH ≥ 3 by the high background conductivity at low pH. It is worth mentioning that, even in neutral solutions, chain scission of hyaluronic acid is significantly faster ($k = 500$ s^{-1} at pH 7)[43] than in the case of chitosan, indicating marked differences in the stability of the precursor radicals. On the other hand, chain scission initiated by a β-fragmentation of alkyl radicals in synthetic polyelectrolytes[45,61] is much slower than scission of the glycosidic bond (by hydrolysis and β-fragmentation) in chitosan.

The above data refer to a solution containing no (deliberately added) salt. Upon addition of sodium perchlorate the counterion release slows down ($k \approx 80$ s^{-1} at 5×10^{-4} mol dm^{-3} salt and $k \approx 60$ s^{-1} at 2×10^{-3} mol dm^{-3} salt), while the signal amplitude (scission yield) remains practically unchanged. The reason for this "salt effect" is not yet known.

The final yield of conductivity increase corresponds to the release of *ca.* 1.8 counterions per $^{\bullet}OH$ generated in the system. Assuming that the yield of chain breaks under these conditions is equal to that measured for γ-radiolysis ($G_s = 3.4 \times 10^{-7}$ mol J^{-1}), and that no crosslinking between chitosan chains takes place, the number of counterions released for each chain break is ~3.1. This number is lower than the values of 5–8.5 reported for synthetic vinyl polyelectrolytes and for polynucleotides.[60,61,63] The reason for this difference is most probably the lower linear charge density in the present case (longer distance between the protonated amino groups, presence of non-charged *N*-acetylglucosamine units), leading to a less efficient counterion condensation.

In the above discussion and calculations it was assumed that no intermolecular crosslinking takes place between the chitosan chains as a result of radical recombination (such a process would result in an increase of the average molecular weight). Although polysaccharides are commonly considered as "radiation-degrading" polymers, this is not obvious. With low-molecular-weight, neutral carbohydrates, recombination reactions in deoxygenated solutions lead to the formation of dimers.[64,65] Taking this, and the high yields of scission, into account, one may conclude that recombination of radicals mainly occurs within the same macromolecule (formation of small loops). This would not lead to an increase in the molecular weight, despite the fact that a recombination of radicals has occurred. Loop formation is a well-known process in the radiation chemistry of polymers, and many interesting properties of micro-hydrogels are based on this effect.[13,66–69]

Changes in molecular weight upon γ-irradiation at high (2.9 Gy s^{-1}) and low (0.082 Gy s^{-1}) dose rate indicate that there is no significant difference in the scission yields (data not shown). This deserves a comment. For the high dose rate experiment it can be estimated that at the low dose given (15 s irradiation time) the steady-state concentration of radicals that could build-up at this dose rate is not yet reached, and each macromolecule will contain (on average) about three radicals. This radical density is high enough to induce loop formation.[42] At the low dose rate (data shown in Fig. 3) the steady state is reached [assuming $2k = 250$ dm^3 mol^{-1} s^{-1}, *i.e.* a 50-times faster decay than fully deprotonated poly(acrylic acid) radicals or a 3-times faster decay than poly(methacrylic acid) radicals]. Under this condition, one estimates about one radical per macromolecule. However, one has further to take into account that in ionizing radiation radical-formation occurs in spurs, *i.e.* two or more radicals are always formed in these spatially small areas, wherefrom they diffuse into the bulk solution. There is a certain likelihood that radicals from a given spur react predominantly with one macromolecule, *i.e.* the distribution of radicals at the macromolecules will be not fully Gaussian. Thus, there may be a bias towards a number of macromolecules that have a rather high number of radicals and some that have none at all. If one accepts this view, results from the low-dose-rate and high-dose-rate experiments should not differ too much, as has been found.

Furthermore, formylmethyl radicals formed by water elimination from the 1,2-dihydroxyethyl radical (derived from ethylene glycol) can abstract an H-atom from the substrate, thereby inducing a short chain reaction.[70] However, no chain reactions were observed with monosaccharides under otherwise similar conditions,[50] possibly because in these systems the radicals formed upon water elimination are less reactive secondary radicals. In chitosan, where the radical lifetime becomes very long due to the repulsive forces between the charged chains, such a process could again start to play a role. This could com-

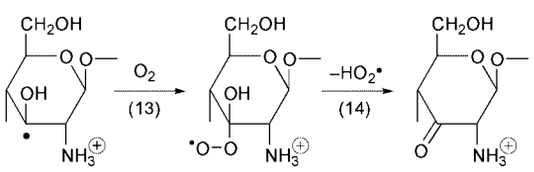
pensate for the dimer formation at low dose rates by inducing new radicals that can subsequently cause scission.

Chain scission in dioxygen-containing solutions

When chitosan is irradiated in oxygenated solution, dioxygen adds to the initially formed carbon-centered radicals forming the corresponding peroxyl radicals [*e.g.* reaction (13)]. This reaction is practically diffusion-controlled.[71] While rate constants in the order of 2×10^9 dm^3 mol^{-1} s^{-1} are typical for low-molecular-weight carbohydrates, the value for chitosan may be slightly lower, since the solubility of dioxygen in the ion-rich condensation layer surrounding the charged chain is lower than in the bulk solution (*cf.* refs. 72 and 73).

The absorption spectra of the chitosan-derived peroxyl radicals (including $HO_2^{\bullet}/O_2^{\bullet -}$, inset in Fig. 2) are similar to those of the parent carbon-centered radicals. This did not allow us to determine the rate of dioxygen addition to the chitosan radicals.

In carbohydrates, the α-hydroxyalkyl peroxyl radicals readily undergo $HO_2^{\bullet}/O_2^{\bullet -}$ elimination.[35,50,74] In our system, this would be the peroxyl radicals at C(2), C(3) and C(6) [*e.g.* reaction (14), the amino group behaves similarly to the hydroxyl group in this respect; note that due to an increased acidity of the ammonium function upon peroxyl radical formation at C(2), such a process can also occur at pH ~ 3, because the ammonium function is expected to be deprotonated (for the Taft s^* value of the –OO$^{\bullet}$ substituent, see ref. 75)].



The formation of $O_2^{\bullet -}$ can be monitored with tetranitromethane by following the formation of the nitroform anion, which absorbs strongly at 350 nm [reaction (15)].[76]

$$O_2^{\bullet -} + C(NO_2)_4 \longrightarrow O_2 + C(NO_2)_3^- + {}^{\bullet}NO_2 \quad (15)$$

The pK_a value of $HO_2^{\bullet}$ is 4.8,[77] but at pH 3, where our experiments have been carried out, the concentration of $O_2^{\bullet -}$ in equilibrium is still sufficient for reaction (15) to proceed at a fast rate, and the yield of $O_2^{\bullet -}$ can be determined by pulse radiolysis. Analysis of the data (not shown) indicates that 55% of the chitosan radicals eliminate $HO_2^{\bullet}/O_2^{\bullet -}$.

Peroxyl radicals at C(1), and C(4) may contribute to chain scission, but our knowledge of the underlying reactions is still too limited (*cf.* ref. 78) for us to come up with a convincing detailed mechanistic scheme. However, in disaccharide model systems and in other carbohydrate polymers it is generally noted that in the presence of dioxygen the scission of the glycosidic linkage is considerably reduced, compared to deoxygenated solutions.

The radiation-chemical yield of scission in N_2O–O_2-saturated solutions is $G_s = 2.1 \times 10^{-7}$ mol J^{-1} (open circles in Fig. 3) as compared to $G_s = 3.4 \times 10^{-7}$ mol J^{-1} in the absence of dioxygen. This protection factor of 1.6 is somewhat lower than the protection factors of 2.4 and 2.3 found for cellobiose[51,78] and for hyaluronic acid,[37,43] respectively. This interesting protecting effect is in some contrast with the usual observation that the presence of dioxygen enhances free-radical induced polymer degradation.

In oxygenated solutions, pulse-radiolytic experiments with conductometric detection on the kinetics of chain breakage are often complicated by the formation of conducting species, notably H^+ and $O_2^{\bullet -}$, in side reactions. In the pH range used in this work this is not a major obstacle, since most of the $O_2^{\bullet -}$ radicals are protonated [p$K_a(HO_2^{\bullet}) = 4.8$].[77] The overall signal

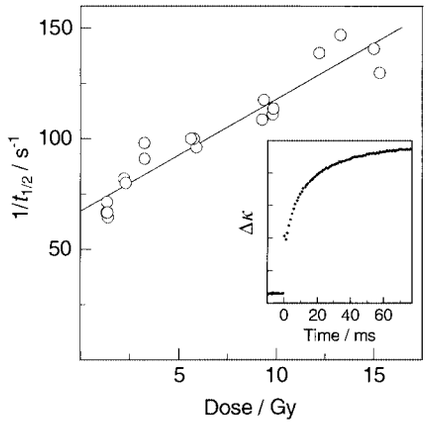


Fig. 5 Pulse radiolysis of N_2O–O_2-saturated aqueous solutions of chitosan (5×10^{-4} mol dm^{-3}), pH 3.3. Reciprocal first half-lives of conductivity increase as a function of dose per pulse (the dose is only known to an accuracy of ±25%, see Experimental section). Inset: A typical trace at ~10 Gy.

amplitude of conductivity increase observed in a N_2O–O_2-saturated solution of pH 3.3 is *ca.* 65% of the value recorded in the N_2O-saturated solution under identical conditions. This is in fair agreement with the ratio between the yields of chain scission upon γ-radiolysis in the presence and absence of dioxygen. Experiments carried out at pH 3.3 (Fig. 5) indicate that scission in the presence of dioxygen occurs by mixed first- and second-order kinetics.

There is also some variation with pH (lower rates at lower pH), but since glycosidic bond scission in the presence of dioxygen is only partly understood even on the disaccharide model level, it is premature to suggest mechanistic implications for the chitosan system.

Concluding remarks

Counterion release accompanying chain scission had only been studied with polyanions so far, and the present study now shows this also for a polycationic system. This supports very well the theory of scission-induced counterion release in polyelectrolytes. The pulse radiolysis data show that the chain scission process is very fast and that it would be very difficult, even at the high dose rates of electron-beam technology, to avoid this process altogether in the design of chitosan-containing composite material involving free-radical-induced processes. On the other hand, efficient OH-radical-induced chain scission is a most welcome, easy and economical means of reducing the molecular weight of chitosan by the irradiation of its aqueous solution.

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