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Degradation of chitosan and starch by 360-kHz ultrasound

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Abstract

Effects of 360-kHz ultrasound on aqueous solutions of chitosan and starch were studied. This treatment is an efficient procedure for reduction of molecular weight of both polysaccharides. It has been demonstrated that at the applied ultrasound frequency degradation is caused both by OH radicals and mechanochemical effects. In Ar-saturated 2×10^{-2} mol dm^{-3} chitosan solutions, pH 3, at an ultrasound dose rate of 170 W kg^{-1}, the average sonochemical chain scission yield in the sonication time range of 0–90 min is ca. 8×10^{-11} mol J^{-1}. This yield has been shown to depend on polymer concentration, ultrasound power and gas used to saturate the solution. Scission is accompanied by side reactions leading to the formation of carbonyl groups. Ultrasound-induced chain scission of starch proceeds with lower yield, one of the reasons being probably the different chain conformation when compared with rod-like chitosan macromolecules.

Keywords: Ultrasound; Sonochemistry; Degradation; Chitosan; Starch; Hydroxyl radical; Radical reactions; Mechanochemical reactions

1. Introduction

Numerous properties of polysaccharides depend on their molecular weight. This refers both to simple physical characteristics as solution viscosity and to more complex properties like biological activity. For example, there is an increasing evidence that the suitability of chitosan for a particular pharmaceutical or biological application and its effectiveness in exerting a specific action, such as fat-binding, antithrombotic activity or stimulating plant growth depends on its molecular weight (Allan et al., 1984; Ikeda et al., 1993; Inui, Tsujikubo, & Hirano, 1995; Meler, Pluta, Ulanski, & Krotkiewski, 2003; Muzzarelli, Trebojevich, & Cosani, 1996; Rosiak, Ulanski, Kucharska, Dutkiewicz, & Judkiewicz, 1992; Torzsas, Kendall, Sugano, Iwamoto, & Rao, 1996).

Since the average chain length of natural polysaccharides is determined by their source of origin and cannot be, in most cases, influenced, there is a need for fast, efficient and inexpensive methods of processing the native polysaccharide substrates in order to achieve the desired average molecular weight (or, in same cases, also a desired molecular weight distribution). Chemical treatment, for example acid hydrolysis, is often time- and energy consuming, involves use of chemicals and generates waste. Moreover, being a multi-parameter process, it is not always easy to control, although considerable progress in this technique has been achieved (Allan & Peyron, 1995a, b; Hasegawa, Isogai, & Onabe, 1993; Wojtasz-Pajak, Kolodziejska, Debogorska, & Malesa-Ciecwierz, 1998). Also the enzymatic processing is a relatively complex procedure (Nordtveit, Varum, & Smidsrod, 1994; Varum, Holme, Izume, Stokke, & Smidsrod, 1996; Wojtasz-Pajak et al., 1998).

Two alternative polysaccharide degradation methods are: irradiation (i.e. treatment with ionizing radiation) and sonication (treatment with ultrasound), cf. (Lorimer, Mason, Cuthbert, & Brookfield, 1995; Ulanski, Wojtasz-Pajak, Rosiak, & von Sonntag, 2000b). Irradiation is the simplest way to degrade a polysaccharide in any physical form (solid, suspension, paste, solution, etc.). The process is very efficient and can be easily controlled by choosing a proper irradiation dose. No chemicals are needed, there is no

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additional processing and there is no waste. Besides, at appropriate conditions sterilization may be accomplished in parallel to the reduction in molecular weight. A disadvantage that may limit the applicability of solid-state irradiation is the possibility of some radicals being trapped in crystalline regions of polymer, resulting in further degradation (‘post-effect’), slow but difficult to control (Rosiak et al., 1992; Ulanski & Rosiak, 1992). Another factor that may discourage from the application of this technique is that suitable radiation sources or radiation-processing plants are not always readily available.

Degradation with ultrasound seems to be a promising alternative. It is usually performed in solution (aqueous, in case of polysaccharides), although suspensions can also be treated.

When a liquid is subjected to the action of ultrasound, each volume element experiences variations in pressure. If the temporary reduction of pressure falls below the threshold of tensile strength of the liquid, a rupture in the liquid occurs in a form of a small bubble filled with vapour (and gas, if present in the liquid as a solute, as well as molecules of any other volatile solutes). The formation of these bubbles and their subsequent collapse is called cavitation.

Some cavities exist only for one cycle of the sound field and collapse violently (transient cavities), while other are long-lived and oscillate around some equilibrium size (stable cavities). Cavitation is the primary effect that in consequence leads to the initiation of chemical reactions in the system.

Rapidly collapsing cavitation bubbles induce high pressure gradients and high local velocities of liquid layers in their vicinity. This in turn may cause shear forces that have no significant influence on small molecules, but are capable of breaking the chains of polymers, provided the chains are longer than a certain limiting value. This is the mechanochemical action of ultrasound on polymers.

Because of the high frequency of ultrasound, the implosive collapse of a bubble is an adiabatic process that leads to a rapid, momentary temperature increase up to ca. 5000 K in the gas phase of the collapsing bubble and well above 1000 K in the thin layer of liquid adjacent to the cavity (Flint & Suslick, 1991; Suslick, Hammerton, & Cline, 1986).

Solvent molecules, abundant in the collapsing bubble, may dissociate to form radicals. Water is partially decomposed into OH radicals and H atoms. Some of these radicals diffuse out of the cavity to the surrounding liquid, where they can react with solute molecules. This mechanism makes sonochemistry similar, in a sense, to radiation chemistry, where at first solvent radicals are generated, that subsequently attack the solute (von Sonntag, Mark, Tauber, & Schuchmann, 1999).

Besides these two mechanisms (mechanochemistry and radical attack), polymers in solution may undergo pyrolysis in the hot interfacial region between the bubble and the surrounding liquid.

In the case of polymers subjected to ultrasound in solution, all the above mentioned mechanisms may be operative. Very broad evidence has been gathered for the occurrence of the shear-induced polymer degradation. A characteristic feature of this process, being in contrast with other degradation mechanisms, e.g. the radiation-induced chain scission, is that it proceeds in a non-random manner (breakage near the mid-point of the chain is preferred), and that there is a definite minimum chain length limiting the degradation process. When it is reached, no further chain scission is observed (Basedow & Ebert, 1977; Henglein & Gutierrez, 1988; Malhorta, 1982, 1986; Price & Smith, 1993; Price, West, & Smith, 1994). Somewhat less is known about the OH-radical-induced degradation (Alexander & Fox, 1954; Henglein, 1995), that is expected to be of importance mainly in the 100–1000 kHz frequency range, where the sonochemical yield of OH reaches its maximum (Tauber, 1998; von Sonntag et al., 1999). Pyrolysis, as a local phenomenon limited to the surface of the cavitation bubbles, is of considerable importance mainly in the case of relatively hydrophobic solutes (Henglein et al., 1988; Rokita & Ulanski, 2005) and it is not expected to play a dominant role in the sonochemical degradation of chitosan or starch.

With the advent of ultrasonic reactors capable of providing controllable and adjustable reaction conditions (frequency, ultrasound power, temperature, gas saturation, continuous vs. pulsed sonication), it became easier to undertake qualitative and quantitative studies on sonochemical reactions in polymer solutions. It seems that the high-frequency range, where both mechanochemical and radical-induced reactions take place, is particularly interesting to explore (Tauber, 1998; von Sonntag et al., 1999).

While the chemistry underlying radiation-induced degradation of polysaccharides has been extensively studied in the past and the basic reaction mechanisms are described in detail (Phillips, 1980; von Sonntag, 1980, 1987), much less is known on the mechanisms of ultrasound-induced chain scission in aqueous solution. Positive exceptions from this general picture are detailed and informative works on the mechanism of sonolysis of dextran (Lorimer et al., 1995; Portenlaenger & Heusinger, 1997). In most published works on chitosan and starch (in the case of starch first report dates from 1930s: (Szalay, 1933); for a brief review on the sonochemistry of carbohydrates, see (Kardos & Luche, 2001); recent works on chitosan are: (Chen, Chang, & Shyur, 1997; Chen & Chen, 2000; Chen & Hwa, 1996; Signini, Desbieres, & Campana Filho, 2000; Tang, Huang, & Lim, 2003; Trzcinski, Staszewska, & Bohdanecky, 2001)) degradation is evidenced, but detailed mechanistic considerations are rarely undertaken. A general picture attributing the degradation to purely mechanochemical effects is not necessarily complete, at least when high ultrasound frequencies (hundreds of kHz) are applied.

A factor that does not contribute to the clarity of the picture is that in sonochemical studies, in contrast to radiation chemistry, there is no universally accepted method of quantifying the experimental data, both in the terms of ultrasound energy absorbed and the yields of products as well as kinetic models. Thus, the experimental data reported in one paper more often than not cannot be compared with results published in other works. Also experimental conditions vary (solvent composition, temperature, gas saturation, etc.). Moreover, results published by various authors describe sonochemical processes caused by ultrasound waves of different frequencies, and it is known that not only yields, but also the mechanisms involved are strongly frequency-dependent.

In our work we certainly do not intend to clarify all these discrepancies. However, we try to provide, at least for the main object of this study—chitosan, quantitative results in the terms analogous to those used in radiation chemistry, which are being recently more often used in sonochemistry as well (von Sonntag et al., 1999).

The aim of this work is to provide quantitative data on the ultrasound-induced degradation of chitosan in aqueous solution, at an ultrasound frequency where two effects—mechanochemistry and radical reactions—are expected to contribute to chain scission. We also wanted to provide experimental indications confirming the existence of both these mechanisms. Data on sonochemical degradation of starch at identical conditions were provided for comparison. Furthermore, our aim was to explore and explain the influence of polymer concentration, ultrasound power and gas used for saturation on the yields of chain scission.

2. Materials and Methods

Chitosan from crab shells was custom-synthesized at the Sea Fisheries Institute (Gdynia, Poland), (Ulanski et al., 2000b; Wojtasz-Pajak et al., 1998). The molecular parameters were: weight-average molecular weight 323 kDa (determined by multi-angle laser light-scattering, see below) and degree of deacetylation ca. 88% (as estimated by the potentiometric titration method (Tolimate et al., 2000; Wojtasz-Pajak et al., 1998)). Corn starch was supplied by Sigma. All other chemicals were of reagent grade and were used as received.

Aqueous solutions were made up in water purified by Nanopure II system (Barnstead, USA). Sonications were performed in a URS-1000 ultrasonic reactor setup (Allied Signal Elac-Nautik, Kiel, Germany), consisting of a CESAR wave generator and amplifier, ultrasonic transducer and a thermostated cylindrical reactor of 500 ml capacity. The vibrating element of the transducer, covered with stainless steel, formed the bottom of the reactor. The reactor was filled with water, and the sample (10 ml) was contained in a small gas-tight, flat-bottom glass vessel placed at a fixed position in the central part of the reactor. Before sonication, the sample was saturated with a suitable gas (Ar, He, O_2). During sonication, the small vessel rotated at ca. 3 rev s^{-1} in order to assure uniform absorption of ultrasound energy. Ultrasound frequency was 360 kHz, the average bulk temperature of sonicated solution was maintained at 22 ± 2 °C. The dose rate of ultrasound absorption, equal 170 W kg^{-1} at the nominal output power of 100 W, was determined by calorimetry (Tauber, 1998).

Weight-average molecular weights were determined by multi-angle laser light-scattering on a BI-SM 200 setup (Brookhaven Instruments) equipped with a Innova 90C argon ion laser (coherent $\lambda = 514.5$ nm). Chitosan was analyzed in solutions of pH 3.0 ($HClO_4$) containing 0.25 mol dm^{-3} sodium perchlorate. The refractive index increment of chitosan in this solvent is $dn/dc = 0.187$ cm^3 g^{-1} at 25.0 °C (Ulanski & von Sonntag, 2000a; Ulanski et al., 2000b). Directly before the light-scattering measurements, chitosan solutions were passed through filters (Minisart, Sartorius) of 0.45 μm pore size. Intensity of scattered light was measured for at least five dilutions of each sample in the angular range 30–120°. Zimm-plot algorithm was applied for the evaluation of results (Kratochvil, 1987). All the presented data points are averages of at least three independent sonication experiments.

Viscosity measurements on starch solutions in 0.05 M $NaClO_4$ were made at a temperature of 25.0 ± 0.1 °C using an AVS-350 setup (Schott Geräte) equipped with an Ubbelohde viscometer. The Mark–Houwink parameters for starch under these conditions are: $K = 1.44 \times 10^{-5}$ cm^3 g^{-1}, $\alpha = 0.93$ (Young, 1984).

UV spectra of chitosan solutions were recorded in 10-mm quartz cells on a Perkin–Elmer Lambda 40 UV/Vis spectrophotometer. Unless stated otherwise, pure solvent (aqueous $HClO_4$, pH 3) was used as a reference.

Unless stated otherwise, chitosan concentrations are given in mol dm^{-3} of the repeating unit (165 Da).

3. Results and Discussion

When chitosan is subjected to the action of 360-kHz ultrasound in an Ar-saturated aqueous solution of pH 3, it undergoes degradation (main chain scission). This manifests itself as a decrease in molecular weight. We followed degradation by direct determination of changes in weight-average molecular weight by multi-angle laser light-scattering (filled circles in Fig. 1)

Based on these data, we calculated the molar concentration of chain break events, using the formula (Charlesby, 1960; Schnabel, 1981)

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

where M_{w0} and M_w is the weight-average molecular weight before and after sonication (in g mol^{-1}) and c is polymer concentration (in g dm^{-3}).

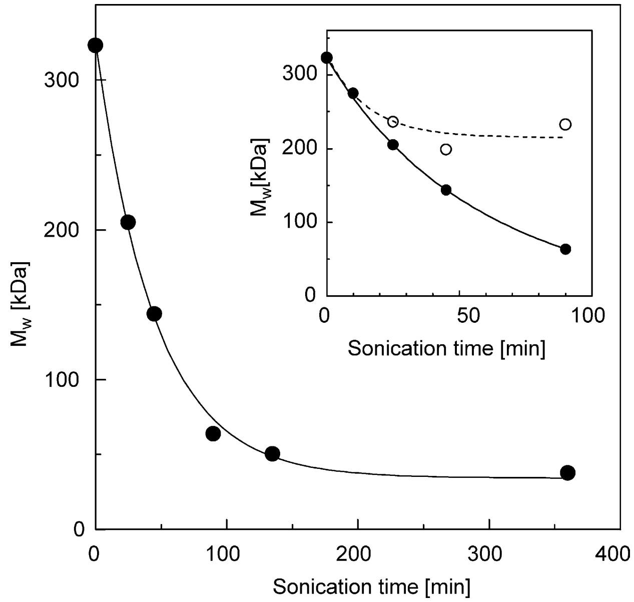

Fig. 1. Sonolysis (360 kHz, nominal power 100 W, dose rate 170 W/kg, 22 °C) of 2×10^{-2} mol dm^{-3} chitosan in Ar-saturated aqueous solution at pH 3.0 ($HClO_4$) in the absence (●) and presence (○) of 5×10^{-3} mol dm^{-3} *tert*-butanol. Weight-average molecular weight as a function of sonication time.

These results are shown in Fig. 2 (filled circles, solid line). Within the investigated time range, the number of broken bonds increases with sonication time, and the slope of this dependency, corresponding to the yield of chain scission, seems to increase in the course of the reaction (for a discussion of the latter effect, see below).

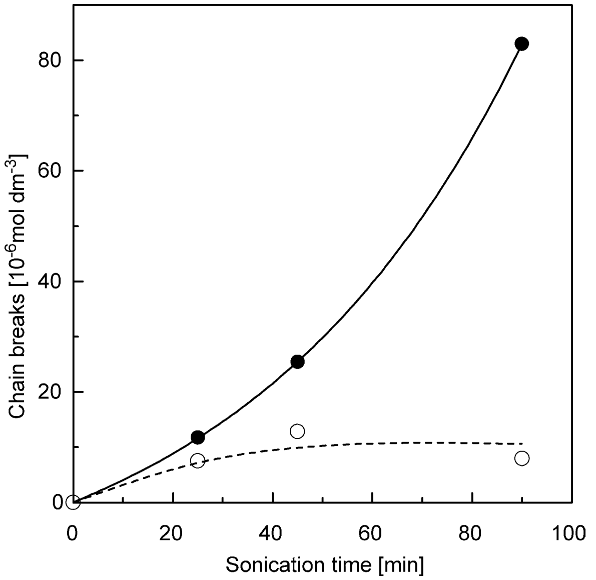

Fig. 2. Sonolysis (360 kHz, nominal power 100 W, dose rate 170 W/kg, 22 °C) of 2×10^{-2} mol dm^{-3} chitosan in Ar-saturated aqueous solution at pH 3.0 ($HClO_4$) in the absence (●) and presence (○) of 5×10^{-3} mol dm^{-3} *tert*-butanol. Concentration of chain breaks of chitosan as a function of sonication time.

Knowing the dose rate of ultrasound absorption (i.e. the speed at which a unit mass of the system absorbed ultrasound energy) to be 170 W kg^{-1}, for each sonication time we were able to calculate the ultrasound dose, defined as the ultrasound energy absorbed by unit mass of the solution (in J kg^{-1}). Number of moles of chain scission events per mass unit of the system divided by the dose is the sonochemical yield of chain breakage (in mol J^{-1}). From the initial slope of solid line in Fig. 2 we calculated the sonochemical yield of chain breakage under the studied conditions as $G_s = 4.6\times10^{-11}$ mol J^{-1}, while the average value in the studied time range is ca. 8.1×10^{-11} mol J^{-1}. This value is significantly lower than the typical yields of OH formation under similar experimental conditions, the latter being in the order of 1×10^{-9} mol J^{-1} (Tauber, 1998; von Sonntag et al., 1999). This is fully understandable, since most of OH radicals, that are formed locally in the cavitation bubbles and are present in the immediate vicinity of these bubbles at high concentrations, undergo mutual recombination ($2k = 1.1\times10^{10}$ dm^3 mol^{-1} s^{-1}; (Buxton, Greenstock, Helman, & Ross, 1988)) before they may reach chitosan molecules, and out of those that react with chitosan only a fraction would yield scission-initiating radicals. Moreover, the OH yield in chitosan solution is probably lower than in pure water since much higher solution viscosity may reduce the intensity of cavitation (see the polymer concentration effect below).

In order to confirm the important role of OH radicals in initiating the sonochemical degradation of chitosan, we repeated the above-described experiments in the presence of small amounts of *tert*-butanol, a very efficient scavenger of OH radicals. At the concentration used (5×10^{-3} mol dm^{-3}) this compound would not influence cavitation, but should strongly reduce the number of hydroxyl radicals available for reaction with chitosan. Efficiency of *tert*-butanol in scavenging OH radicals even at low concentrations in sonochemical experiments results not only from its high reactivity towards OH ($k = 6\times10^8$ dm^3 mol^{-1} s^{-1}; (Buxton et al., 1988)), but also from the ability of the relatively hydrophobic *tert*-butanol molecules to accumulate at the surface of cavitation bubbles, i.e. at the location where hydroxyl radicals are generated (von Sonntag et al., 1999; Tauber, Mark, Schuchmann, & von Sonntag, 1999). Results are shown in Figs. 1 and 2 as open circles and broken lines. Despite some scatter in data, inevitable in this multi-component, non-homogeneous system, it can be clearly seen that chitosan degradation in the presence of millimolar concentrations of *tert*-butanol is much less pronounced than in its absence. Moreover, the reaction becomes gradually less efficient with sonication time, what is expected for mechanochemical effects. Only long chains are vulnerable to breakage by the action of hydrodynamic forces, while chains below some limiting critical size cannot be degraded in this way (in an earlier work on chitosan (Chen et al., 2000), this limiting size, presumably under pure mechanochemical conditions, has been estimated as ca. 100 kDa). With decreasing molecular weight the yield of this process should be going down, which is in fact observed.

As we see in Fig. 2, in the presence of *tert*-butanol the yield of chain scission drops down to the range of 2.9×10^{-11} mol J^{-1} (initial value) to ca. 1.4×10^{-11} mol J^{-1} (mean value in the measured time range). Based on the initial values, scission in the presence of an OH scavenger amounts to ca. 60% of the value observed in its absence. From these data it comes out that mechanochemical reactions do not account for more than ca. 60% of the chain breaks generated in chitosan under our initial conditions. The actual number may be even lower, since some fraction of OH radicals may still have escaped scavenging by *tert*-butanol. With the progress of scission, for shorter polymer chains, the fraction of mechanochemical effects in the degradation seems to drop even below 20%.

For OH-radical-induced degradation, a constant or slightly increasing yield should be expected. Hydroxyl radicals react with chitosan by hydrogen abstraction. The rate constant of this reaction, measured as $k=6.4\times10^{8}$ dm^3 mol^{-1} s^{-1} for chitosan similar to our starting material (Ulanski et al., 2000a), is expected to increase with decreasing chain length (for detailed studies on this effect, see e.g. (Matheson, Mamou, Silverman, & Rabani, 1973; Ulanski, Zainuddin, & Rosiak, 1995)). Therefore, in the course of degradation, reaction of hydroxyl radicals with shorter chitosan molecules may more efficiently compete with OH self-combination. The observed trend in the chain scission yield for a scavenger-free solution (filled circles and solid line in Fig. 2) is in line with this expectation.

The OH attack on a chitosan molecule occurs at random position and, as a result, transient, unstable radicals are generated at the chitosan chain. Some of them, in particular those located at C_1, C_4 and C_5 positions, may undergo transformation, mainly by hydrolysis and fragmentation, leading to the breakage of 1–4 glycosidic bond (Ulanski et al., 2000a; von Sonntag, 1980, 1987). The overall rate constant of chain scission in chitosan in aqueous solution has been determined previously as $k\approx95\ s^{-1}$ (Ulanski et al., 2000a).

Fig. 3 illustrates the influence of chitosan concentration on the final molecular weight of samples that have absorbed the same ultrasound dose (equal sonication time at the same dose rate). In inset, the same data are recalculated into chain scission yields. The yield decreases with increasing polymer concentration. This most probably reflects the increase in viscosity of the solution, as a parameter that may reduce the intensity of cavitation.

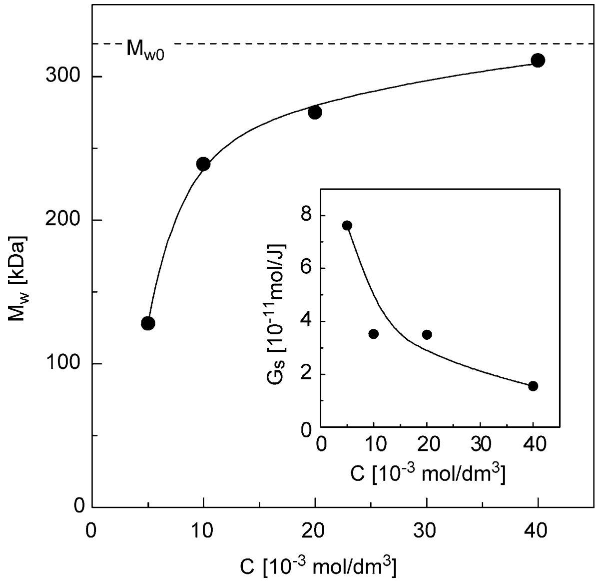

Fig. 3. Sonolysis of chitosan (10 min, 360 kHz, nominal power 100 W, dose rate 170 W/kg, 22 °C) in Ar-saturated aqueous solution at pH 3.0 ($HClO_4$). Weight-average molecular weight after sonication as a function of chitosan concentration. Broken line denotes the starting molecular weight. Inset: yield of chain scission as a function of chitosan concentration.

It would be also of interest to see what is the influence of ultrasound power (or, in other terms, the dose rate) on the efficiency of degradation. In order to study this effect, 2×10^{-2} mol dm^{-3} chitosan solutions, Ar-saturated, have been submitted to the action of ultrasound at various nominal ultrasound powers (the powers of 20–100 W correspond to the dose rates of 34–170 W kg^{-1}). The sonication time was inversely proportional to the power, so that always a constant amount of energy (constant dose) was absorbed. In Fig. 4 the final values of weight-average molecular weight are shown, while inset in this figure illustrates the power dependence of the degradation yield.

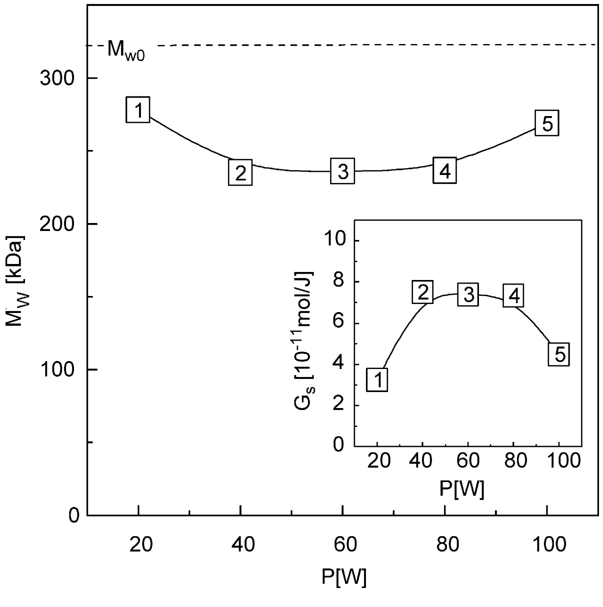

Fig. 4. Sonolysis (360 kHz, 22 °C) of 2×10^{-2} mol dm^{-3} chitosan in Ar-saturated aqueous solution at pH 3.0 ($HClO_4$). Weight-average molecular weight of chitosan as a function of the nominal power of ultrasound. Sonication time was: 1–50 min, 2–25 min, 3–16 min 40 s, 4–12 min 30 s, 5–10 min (constant ultrasound dose equal 102 kJ kg^{-1}). Inset: yield of chain scission as a function of nominal power of ultrasound.

It can be seen that at low power degradation is not efficient. This can be attributed to the fact that in order to induce cavitation, the ultrasound power must reach some minimum value and the lowest power used in this experiment may be close to that threshold. In the dose range of 40–80 W the degradation yield is stable, while it seems to decrease again at the highest power of 100 W. It is known that at high power the efficiency of sonochemical processes may be diminished, since the cavitation bubbles present at high concentration may scatter the ultrasound wave. It is not excluded that at 100 W we already see an onset of such effect.

It has been shown in some studies on physical and chemical action of ultrasound in liquids that the efficiency of processes originating from the rapid compression of cavitation bubbles and the resulting transient high temperatures depends strongly on the nature of the dissolved gas (Hart & Henglein, 1987; Mark et al., 1998; Mead, Sutherland, & Verrall, 1976; Price et al., 1993; Tauber, 1998; von Sonntag et al., 1999). The cavitation bubbles in gas-saturated liquid at moderate bulk temperatures (far below the boiling point of the solvent) are filled mostly with the molecules of dissolved gas and only a small fraction of solvent molecules. Therefore, the physical properties of this gas (if we exclude the potential decomposition of multiatomic gas molecules) determine the effect of bubble compression. In an adiabatic process, the temperature rise when a gas bubble is compressed from a volume V_0 to $V_{\min}$ depends on the C_p/C_v ratio (Eq. (2)). This ratio is higher for monoatomic than for diatomic gas molecules.

$$T_{\max} = T_0 \times \left(\frac{V_0}{V_{\min}}\right)^{C_p/C_v - 1} \tag{2}$$

This is, however, not the only physical factor influencing the results. Heat conductivity is at least of equal importance. If the dissipation of energy during the compression phase is too fast, the process is no longer adiabatic, the heat is transferred to the surrounding liquid and high temperatures necessary to induce the chemical reactions inside the bubble are not reached. This is the probable reason why helium (of heat conductivity equal 14.30×10^{-2} W m^{-1} K^{-1} in comparison to 1.73×10^{-2} W m^{-1} K^{-1} for Ar) usually performs much worse than argon (Mark et al., 1998; von Sonntag et al., 1999). This is confirmed also in our study. The reduction in molecular weight in He-saturated solution is much less pronounced than in Ar-saturated solution, under otherwise identical conditions (Fig. 5). This difference is also reflected in the degradation yields (Fig. 6).

Oxygen has even lower heat conductivity than argon (1.64×10^{-2} W m^{-1} K^{-1}), but this gain is compensated by lower C_p/C_v ratio (1.39 when compared to 1.67 for Ar). Both these gases have also similar solubility in water. High degradation yields in oxygen-saturated solutions must therefore be caused by chemical rather than physical effects. Although in many polymeric systems where degradation proceeds via radical mechanism the presence of oxygen may enhance scission, this effect is usually not observed in polysaccharides (von Sonntag, 1987). In some cases, including chitosan, oxygen may even exert a mild protective effect reducing the degradation yield, as has been observed in the case of radiation-induced degradation of chitosan in aqueous solution (Ulanski et al., 2000a). Therefore, in the present case reaction of oxygen with chitosan radicals

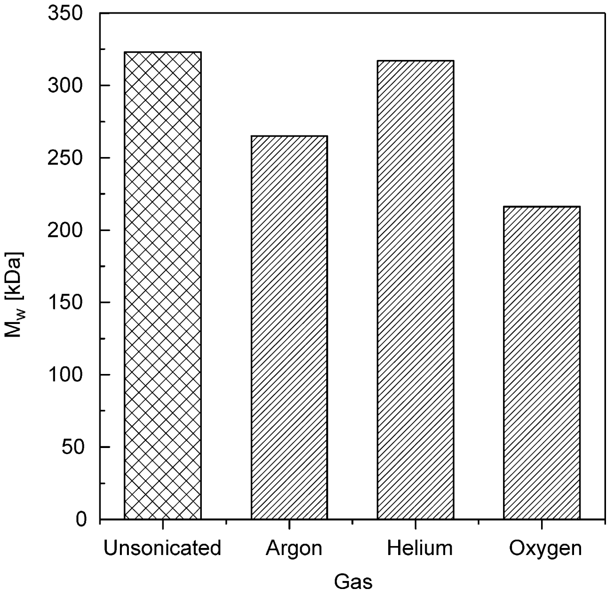

Fig. 5. Sonolysis (10 min, 360 kHz, nominal power 100 W, dose rate 170 W/kg, 22 °C) of 2×10^{-2} mol dm^{-3} chitosan in aqueous solution at pH 3.0 ($HClO_4$). Influence of gas on the final weight-average molecular weight of chitosan.

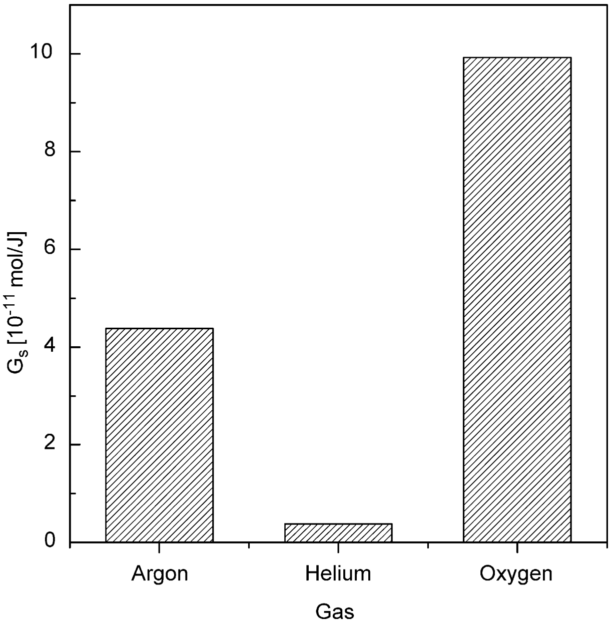

Fig. 6. Sonolysis (10 min, 360 kHz, nominal power 100 W, dose rate 170 W/kg, 22 °C) of 2×10^{-2} mol dm^{-3} chitosan in aqueous solution at pH 3.0 ($HClO_4$). Influence of gas on the yield of chain scission.

cannot be evoked as the reason for the observed high yields. It is known that oxygen, due to its decomposition at temperatures over 2500 K, may influence the mechanism of radical generation in the cavitation bubbles (Hart et al., 1987; Mark et al., 1998; Price et al., 1993; von Sonntag et al., 1999). It is not excluded, that the yield of hydroxyl radical is higher in oxygen-saturated than in argon-saturated solutions. For example, higher sonochemical yields of H_2O_2 in O_2-saturated water have been reported when compared to Ar-saturated water under identical conditions (Mead et al., 1976). It is, however, not clear, to what extent this effect is caused by recombination of hydroxyl radicals or by generation of hydrogen peroxide by other mechanisms. One may also consider a potential enhancement effect of oxygen on the pyrolysis reactions, which in Ar-saturated chitosan solutions are not expected to be of high importance. At present, it is difficult to assess the potential contributions of these mechanisms to the observed increase in degradation yield in oxygenated solutions.

Ultrasound-induced degradation of chitosan in Ar-saturated solutions, both caused by OH radicals or mechanochemical effects, is expected to be accompanied by side reactions, cf. (Ulanski et al., 2000a; von Sonntag, 1987). One source of such processes is a terminal radical formed as a result of glycosidic bond breakage. In the case of OH-mediated process, there are also non-terminal radicals located along the chain, which may be not capable of causing chain breakage, but may undergo other reactions. Some transformations of chitosan-derived radicals may lead to the formation of carbonyl groups. The latter effect, observed also in case of radiolysis of chitosan (Ershov, Isakova, Rogozhin, Gamzazade, & Leonova, 1987; Rosiak et al., 1992; Ulanski et al., 1992), can be followed by UV–Vis spectroscopy. Evolution of absorption spectra of chitosan solutions upon the action of ultrasound is shown in Fig. 7. Two absorption bands, absent in the starting material, build up, at 265 and at 297 nm, i.e. in the typical range for absorption of carbonyl groups. Time dependencies of absorbance at both wavelengths (Fig. 8) show an upward curvature, which may either reflect the somewhat increasing efficiency of OH reaction with chitosan upon the reduction of molecular weight or indicate that at least in part the formation of carbonyl groups is a secondary reaction, involving primary products of sonolysis as substrates.

What is interesting, if chitosan sample is sonicated in the presence of 5×10^{-3} mol dm^{-3} *tert*-butanol, only one peak, at ca. 290 nm, is formed (inset in Fig. 7). It seems to indicate that the formation of the 265 nm band is solely caused by a transformation of non-scission-type radical formed upon OH attack, while the 290–300 nm band might be due to a terminal carbonyl structure resulting from the OH- or mechanochemically induced scission of glycosidic bond. More detailed end-product analysis is needed to elucidate the side reaction pathways and product structures.

Our data on carbonyl formation are in some contrast with an earlier report (Tang et al., 2003) where no chemical changes accompanying sonochemical scission of chitosan chains were detected. This discrepancy may be partially due to a difference in frequencies (20 kHz in the earlier work), dose range and in the detection techniques (IR vs. UV–Vis).

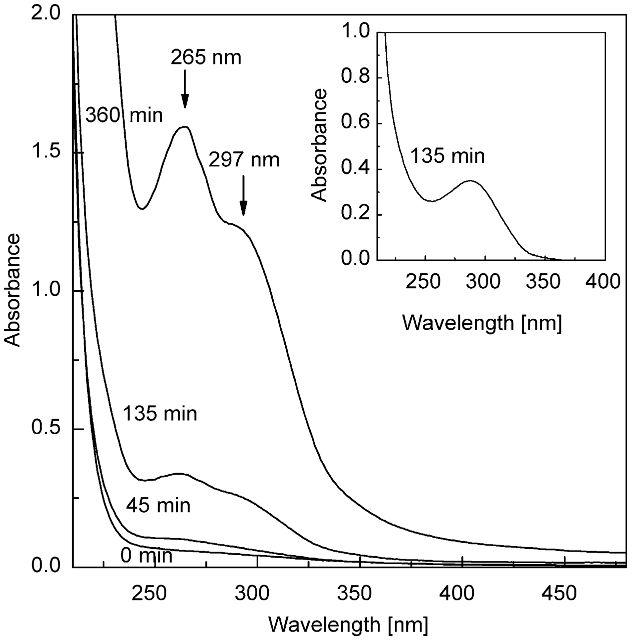

Fig. 7. Sonolysis (360 kHz, nominal power 100 W, dose rate 170 W/kg, 22 °C) of 2×10^{-2} mol dm^{-3} chitosan in Ar-saturated aqueous solution at pH 3.0 ($HClO_4$). Absorption spectra of chitosan solutions, measured for 2×10^{-2} mol dm^{-3} chitosan solution at pH 3.0 ($HClO_4$), at various sonication times. Inset: spectrum of chitosan solution sonicated in the presence of 5×10^{-3} mol dm^{-3} *tert*-butanol (5×10^{-3} mol dm^{-3} aqueous *tert*-butanol solution, pH 3.0, sonicated under identical conditions, was used as a reference).

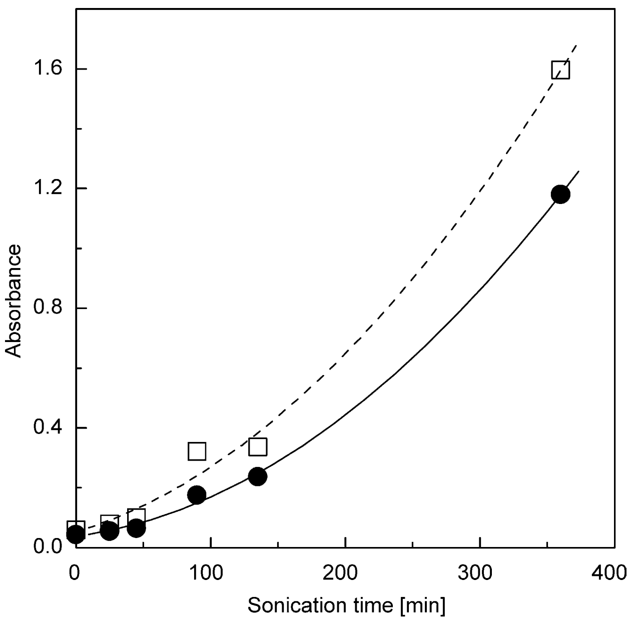

Fig. 8. Sonolysis (360 kHz, nominal power 100 W, dose rate 170 W/kg, 22 °C) of 2×10^{-2} mol dm^{-3} chitosan in Ar-saturated aqueous solution at pH 3.0 ($HClO_4$). Increase in absorbance at $\lambda=265$ nm (☐) and $\lambda=297$ nm (●).

Sonication effects observed for chitosan are not necessarily expected to be identical for other polysaccharides. Although basic OH-induced reactions should be similar in case of most carbohydrate polymers, some qualitative and quantitative differences resulting from different chemical and physical properties may affect both OH-induced and mechanochemical chain scission. The efficiency of the latter depends not only on the chain length, but also on the chain conformation. Chitosan, along with other carbohydrate polyelectrolytes, has a relatively stiff, linear, rod-type conformation due to the repulsive Coulombic forces acting between the charges on chain segments, while neutral polysaccharides are characterized by more coiled conformation. Also the forces between water molecules and polymer chain, contributing to the mechanochemical chain breakage, may be different in these cases. There may be also differences in breakage efficiency between linear and branched and/or partially cross-linked chains.

For comparison, we made measurements on the action of ultrasound on starch solutions, at the same conditions as used for chitosan. Since the starting material had a weight-average molecular weight of over 15 MDa, we were not able to make precise measurements of M_w with the light-scattering technique and therefore we used viscometry to obtain the viscosity-average molecular weight M_v. In the calculations of chain scission yields we assumed $M_w = M_v$. This assumption as well as the necessity of using calculations based on empirical Mark-Houwink parameters instead of absolute light-scattering measurements may cause the starch data to be less accurate than the results obtained for chitosan. Changes in intrinsic viscosity and M_v caused by sonication of starch in Ar-saturated solutions are shown in Fig. 9, while Fig. 10 illustrates the concentration of chain breaks as a function of sonication time.

Similarly as in the case of chitosan, the efficiency of ultrasound-induced chain scission is lowered in the presence of *tert*-butanol. This indicates that at the conditions of our experiments degradation of starch is caused both by the attack of OH radicals and mechanochemical effects. The average yields of chain scission in the studied time range are 2.9×10^{-12} and 2.0×10^{-12} mol J^{-1} in the absence and presence of OH scavenger, respectively.

The scission yields are significantly lower than in the case of chitosan, despite lower solution viscosity (chitosan solutions are more viscous because of its polyelectrolyte character and rod-like conformation; increase in solution viscosity can reduce the intensity of cavitation, see above). For OH-induced reactions this may result from two effects. One is the extremely high average molecular weight of amylopectin, the main starch component, which leads to a low rate constant of OH attack (see the discussion above), and to higher losses of OH due to self-combination. Other reason may be that if OH radical reacts with side chains (absent in chitosan, but present in amylopectin), the reduction of the viscosity-average molecular weight would be much less pronounced than expected for a main-chain attack on a linear macromolecule. Also amylose, due to its much lower molecular weight compared to amylopectin, may effectively scavenge OH radicals with little effect on the viscosity which is determined mainly by the molecular size of amylopectin. Lower mechanochemical scission yield when compared to chitosan is most probably due to

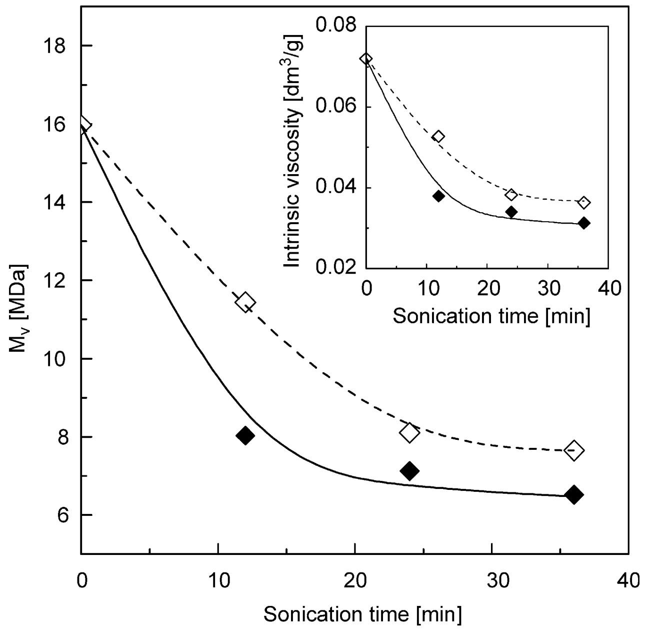

Fig. 9. Sonolysis (360 kHz, nominal power 100 W, dose rate 170 W/kg, 22 °C) of 5 g dm^{-3} starch in aqueous solution containing 5×10^{-2} mol dm^{-3} $NaClO_4$, in the absence (♦) and presence (◇) of 5×10^{-3} mol dm^{-3} *tert*-butanol. Intrinsic viscosity (inset) and viscosity-average molecular weight (main figure) of starch as a function of sonication time.

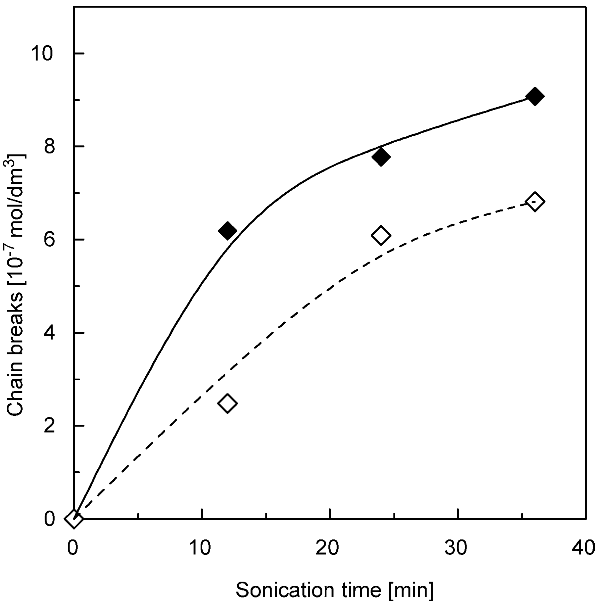

Fig. 10. Sonolysis (360 kHz, nominal power 100 W, dose rate 170 W/kg, 22 °C) of 5 g dm^{-3} starch in aqueous solution containing 5×10^{-2} mol dm^{-3} $NaClO_4$, in the absence (♦) and presence (◇) of 5×10^{-3} mol dm^{-3} *tert*-butanol. Concentration of chain breaks as a function of sonication time.

the difference in conformation. It seems that chains of linear conformation are easier to break, since they may accumulate the 'pulling forces' of the same spatial orientation on much longer distances along the chain than in case of flexible, coil-shaped macromolecules.

Comparison of the average scission yields for starch in the absence and presence of OH-scavenger results in an average estimated fraction of mechanochemical reactions of ca. 70%. It is probably the domination of mechanochemical reactions over all the studied dose range that causes the break vs. time curve for scavenger-free solution to be bent downwards, despite some expected increase in the reaction rate constant of starch components with OH radical (the latter effect can be seen in the chitosan data above).

4. Conclusions

It has been demonstrated on chitosan and starch that treatment with 360 kHz ultrasound in aqueous solution is an efficient procedure for reduction of molecular weight of polysaccharides. Under these conditions, degradation is caused by OH radicals and mechanochemical effects. In Ar-saturated 2×10^{-2} mol dm^{-3} chitosan solutions, pH 3, at a nominal ultrasound power of 100 W (actual dose rate: 170 W kg^{-1}) the average chain scission yield in the time range of 0–90 min has been calculated as ca. 8×10^{-11} mol J^{-1}. Sonochemical yield of this process has been shown to depend on polymer concentration, ultrasound power and gas used to saturate the solution. Scission is accompanied by side reactions leading to the formation of carbonyl groups. Ultrasound-induced chain scission of starch proceeds with lower yields, one of the reasons being probably the different chain conformation when compared with rod-like chitosan macromolecules.

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