Available online at www.sciencedirect.com
SCIENCE @ DIRECT®

Carbohydrate Polymers 56 (2004) 489–498

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Kinetics of ultrasonic degradation and polymerisation degree distribution of sonochemically degraded chitosans

Stanisław Trzciński*, Danuta U. Staszewska

Faculty of Chemistry, Nicholaus Copernicus University, 7 Gagarin Street, 87-100 Toruń, Poland

Received 16 September 2003; revised 6 February 2004; accepted 26 March 2004

Abstract

The process of physical degradation by means of the ultrasonic action towards chitosans with mole fraction of 2-acetamido-2-deoxy-β-D-glucopyranose units (the degree of *N*-acetylation, F_A) in the range of $0.10 \le F_A \le 0.28$, and the weight average polymerisation degree ($\bar{x}_w$) in the range of $2.2 \le \bar{x}_w \times 10^{-3} \le 3.1$ has been investigated. The decrease of $\bar{x}_w$ as well as changes in the distribution of the degree of polymerisation (P) has been determined as a function of time, F_A, temperature, concentration of chitosan solution and concentration of acetic acid in the solution. The use of low-power ultrasound emitter allowed to establish that in the case of chitosan (binary heteropolysaccharide) the general rate parameter (k) increased with F_A. This can be explained by the relatively stronger aggregation of macromolecules with higher F_A, which results in size increase of macromolecular individuals and hence in their higher susceptibility to ultrasonic action. It was also observed that k decreased with chitosan concentration and temperature. The value of limiting degree of polimerisation (x_e) was found to be influenced by structural parameters of chitosan chains (F_A, aggregation). The increase of acetic acid concentration caused the increase in the k value, what indicated accelerating effect of ultrasound towards acidic hydrolysis of chitosan. The shape of the P curve of sonochemically degraded chitosans are in good correlation with the mid-point breakage concept of degradation accepted in sonochemical degradation of polymers.

Keywords: Chitosan, sonochemical degradation; Chitosan, molecular structure; Aggregation; Continuous-distribution kinetics

1. Introduction

Chitosan is a linear binary heteropolysaccharide containing 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) and 2-amino-2-deoxy-β-D-glucopyranose (GlcN) repeating units, linked through (1 → 4) glycosidic linkages. Chitosan is mainly obtained by de-*N*-acetylation of chitin, poly(2-acetamido-2-deoxy-β-D-glucopyranose), which may originate e.g. from exoskeletons of crustaceans or from cell walls of some fungi. The solubility of de-*N*-acetylated chitin in dilute carboxylic acid solution is usually reached when the degree of *N*-acetylation defined by the mole fraction of GlcNAc units (F_A) is ≤ 0.5, and such material is considered as 'chitosan' (Roberts, 1992a, 1997).

The weight average degree of polymerisation ($\bar{x}_w$) of chitosans obtained through de-*N*-acetylation of chitin may be as high as 3500 (Rathke & Hudson, 1994) and for some applications, e.g. in medicine, there is a necessity to lower chitosan degree of polymerisation (x), since low molecular weight chitosan and chitooligomers are expected to be responsible for chitosan biological activity (Folkman & Pospieszny, 2001; Suzuki, Suzuki, Tokoro, Okawa, & Suzuki, 1986). Radiolysis (Ulański & Rosiak, 1992), photolysis (Andrady, Torikai, & Kobatake, 1996), acid and alkaline hydrolysis (Roberts, 1992b; Vårum & Ottøy, 2001), enzymatic degradation (Pantaleone, Yalpani, & Scollar, 1992) are the most frequently applied methods for the disruption of β-(1 → 4) glycosidic linkages in chitosan.

Literature data on sonochemical degradation of chitosan are not numerous. Chen, Chang and Shyur (1997) reported the sonolitic treatment of chitosan solutions with extremely high polydispersity index $\bar{P}_w \approx 10$ ($\bar{P}_w = \bar{x}_w/\bar{x}_n$, where $\bar{x}_n$ is the number average degree of polymerisation). The authors established that the rate of sonochemical degradation increased with the decrease of chitosan solution concentration, what is consistent with results for dextrans obtained by Lorimer, Mason, Cuthbert and Brookfield

---

* Corresponding author. Tel.: +48-56-6114552; fax: +48-56-6542477.
E-mail address: stat@chem.uni.torun.pl (S. Trzciński).

0144-8617/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2004.03.017

(1995) and for other high molecular weight compounds (Jellinek & White, 1951).

Temperature dependence of the rate of sonochemical degradation showed (Chen et al., 1997) better effectiveness of sonication in chitosan solutions at lower (4 °C) temperature, comparing to that at elevated (50 °C) temperature. This statement is consistent with the nature of cavitation, which is more effective in the case of lower vapour pressure of the solvent and is connected with the content of solubilised gases (Basedow & Ebert, 1977; Mason & Lorimer, 1988). The influence of the thermodynamic quality of solvent on sonochemical degradation of chitosan was found to be negligible (Chen et al., 1997).

About ten-fold decrease of $\bar{x}_w$ value of chitosan degraded in high frequency (321 kHz) and high power (170 W/kg) acoustic field was observed by Ulański, Wojtasz-Pająk, Rosiak and von Sonntag (2000). The authors concluded that the mechanism of degradation was complex and could be mainly ascribe to radical random scissions, accompanied to some extend with mechanical breakage caused by hydrodynamic and shear forces.

Investigations on sonochemical degradation of other polymers (among them polysaccharides) are numerous and could shed light on degradation of chitosan. Lorimer et al. (1995) e.g. showed that degradation rate constant decreased with dextran solution concentration and temperature, and increased with sonic power applied. Later, Portenlänger and Heusinger (1997) showed that the optimal frequency (resulting in the highest rate of dextran sonolysis) was 35 kHz, and the highest effectiveness of bond breakage was obtained for dextrans with the highest value of $\bar{x}_n$. They also observed the molecular weight limit, below which the degradation of dextrans stopped.

Basedow, Ebert and Foβhag (1978) have found that the thermodynamic quality of solvent remarkably affects the sonochemical degradation rate. These observations are in good correlation with theoretical aspects of sonication and interactions of acoustic field with macromolecules (Basedow & Ebert, 1977; Mason & Lorimer, 1988), where the most probable mechanism of degradation is assumed to be the mid-point (not random) breakage of macromolecules. Mathematical models of ultrasonic degradation have also been shown by some authors (Glynn, van der Hoff, & Reilly, 1972; Kotliar, 1964a,b). Recently (Madras, Kumar, & Chattopadhyay, 2000) the continuous-distribution kinetics has been applied for poly(vinyl acetate) and polystyrene degraded sonochemically, to describe the time evolution of the molecular weight distribution.

In this study the relation between the general degradation rate parameter (k) of ultrasound degradation and molecular characteristics of chitosan degree of N-acetylation, at specified experimental conditions (chitosan solution concentration, temperature, acetic acid concentration) was shown. Sonolytic degradation of chitosan, on the basis of kinetic data and the change of molecular weight distribution of chitosan was discussed.

2. Experimental

Materials. Chitosans from crab shells or antarctic krill α-chitin used in presented work are obtained from Fluka A.G. (Switzerland) (samples with degree of N-acetylation [0.28], [0.25] and [0.10]) and Sea Fisheries Instytute, Gdynia (Poland) (sample with the degree of N-acetylation [0.23]), respectively. The initial values of $\bar{x}_w$ as well as after 7, 15 and 120 min of sonochemical degradation were determined by SEC technique (described later). Other chemicals (HOAc, NaCl) were of p.a. grade from POCh S.A., Gliwice (Poland).

Degradation. Sonication was carried out in low-power ultrasound emitter: Transsonic T420 being characterised by applied frequency equal 35 kHz, and the sonic intensity ~2 W/cm^2 (from the ratio between the output power and the area of the reactor bottom). The reactor was the 100 ml flat-bottom flask with thermostatic jacket. Degradations were carried out in the air atmosphere with constant rate (~100 rpm) of mechanical stirring.

Viscometry. Initial $[\eta]_0$ values were determined as average values from Huggins and Kraemer plot using flow times measured in Ubbelohde type viscometer. The kinetic energy correction was determined and included in calculations of $[\eta]_0$. In order to simplify measurements of viscosity of systems undergoing degradation, values of $[\eta]_t$ and $[\eta]_0$ were calculated by Matthes (1943); Solomon and Ciuta (1962) methods (so-called one point methods). The difference between values of $[\eta]$ calculated by Huggins and/or Kraemer methods and one point methods were as high as 10% in each case.

Size-exclusion chromatography. Analysis of the distribution of x before and after degradation time t was carried out in the Institute of Chemical Fibres in Łódz, Poland. HPSEC system consisted of isocratic pump HP1050, column system: PL-GFC guard, PL-GFC 300 Å, PL-GFC 4000 Å and refractive index detector HP 1047A/viscometric detector Viscotek system. Software PL Caliber GPC, version 5.1 was used for data handling. As the mobile phase (solvent) the system of Rinaudo, Milas and Dung (1993) was used.

Determination of F_A. F_A values were determined by 1DUV method (Muzzarelli & Rocchetti, 1985; Tan, Khor, Tan, & Wong, 1998) and conformed by ^{13}C CP/MAS spectrometry (Ratajska, Struszczyk, Boryniec, Peter, & Loth, 1997).

3. Results and discussion

3.1. Kinetic parameters

On the basis of experimental results Schmid (1940) concluded that the rate of sonochemical degradation dy/dt of a molecule with degree of polymerisation of x, was proportional to the fraction of polymer chain, which exceeded the limiting degree of polymerisation, x_e, i.e.:

$$\frac{\mathrm{d}y}{\mathrm{d}t} = k(x - x_e) \tag{1}$$

where k is a general rate parameter, which depends on the polymer system under investigation. The cited author (Schmid, 1940) simplified kinetic calculations describing the degradation of macromolecules. Starting from Eq. (1) he derived a practical formulae to estimate k :

$$\frac{x_e}{\bar{x}_t} + \ln\left(1 - \frac{x_e}{\bar{x}_t}\right) = -\frac{k}{c}x_e^2 t + \frac{x_e}{\bar{x}_0} + \ln\left(1 - \frac{x_e}{\bar{x}_0}\right) \tag{2}$$

where $\bar{x}_t, \bar{x}_0$ denotes the mean values of x after degradation time of t, and initial mean value of x of the polymer sample, respectively, and c- molar concentration of the polymer. Schmid (1940) did not specified the type of average values of x and, additionally, the change of P is not considered in Eq. (2). In this paper, Eq. (2) has been adopted to viscometric data. Thus, x is expressed in terms of intrinsic viscosity, $[\eta]$ ($[\eta] = K\bar{M}^a; \bar{x} = \bar{M}/M_0$). After substitution and rearrangement one can get:

$$[\alpha^{1/a} + \ln(1 - \alpha^{1/a})] = -\frac{k}{c}\left[\frac{1}{M_0}\left(\frac{[\eta]_e}{K}\right)^{1/a}\right]^2 t + \beta^{1/a} + \ln(1 - \beta^{1/a}) \tag{3}$$

where $\alpha = [\eta]_e/[\eta]_t$, $\beta = [\eta]_e/[\eta]_0$, ($[\eta]_e, [\eta]_0$ and $[\eta]_t$ denotes limiting intrinsic viscosity, initial intrinsic viscosity and intrinsic viscosity after degradation time t, respectively), a and K are the Mark–Houwink–Kuhn–Sakurada (MHKS) coefficients [according to Roberts and Domszy (1982) $a = 0.93$ and $K = 1.81 \times 10^{-5}$ dl/g for chitosans with $280 \leq x_n \leq 3700$ and $F_A \sim 0.2$], and M_0—molecular weight of the repeating unit. The limiting value approached by $[\eta]$ in the course of degradation was read from the plot of $[\eta]_t$ vs t (Fig. 1). It was found, that at the applied experimental conditions, the value of $[\eta]_e$ was reached after about 1 h of ultrasonic treatment.

The dependence of $[\eta]_e$ and k on experimental conditions and molecular characteristics of chitosan is presented in Table 1.

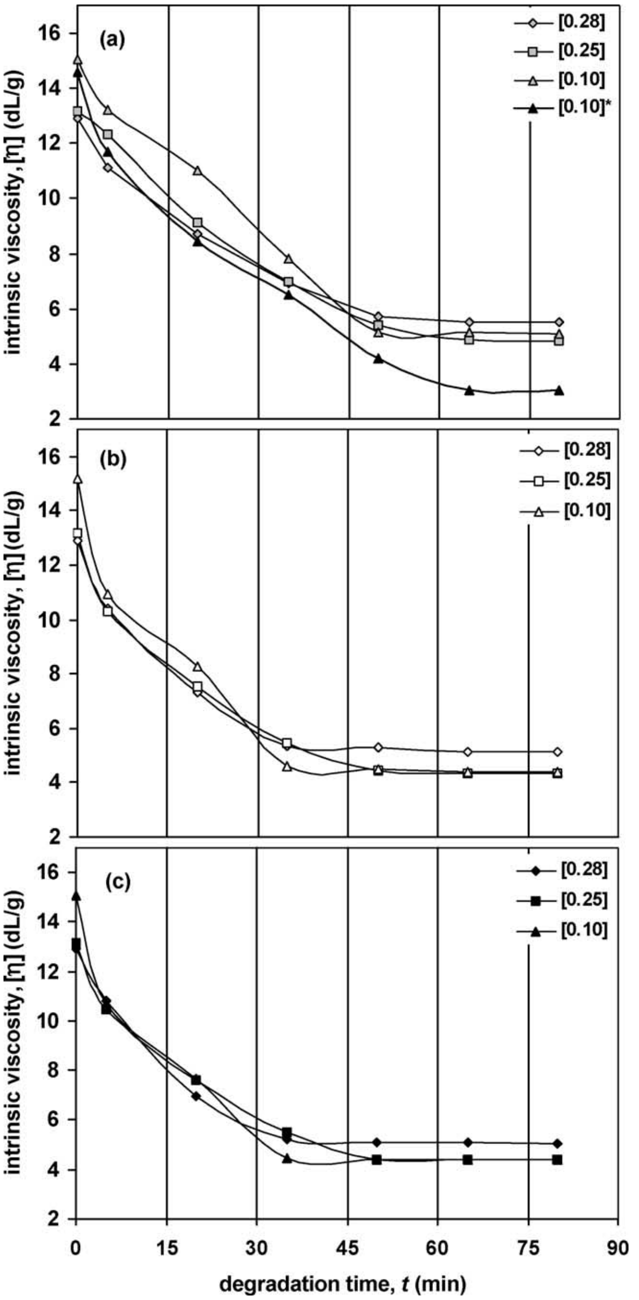

Fig. 1. Kinetic runs for sonochemical degradation of chitosan in 1% (m/v) solution in 0.1 M HOAc ( ) and 1.0 M HOAc (*) at 25 °C(a), 1% (m/v) solution in 0.1 M HOAc at 4 °C (b), and 0.5% (m/v) solution in 0.1 M HOAc at 25 °C(c). F_A values in square brackets.

As it is shown in Table 1, values of $[\eta]_e$ and k depend on molecular structure of chitosan chain and experimental conditions applied during degradation. The highest value of $[\eta]_e$ is observed for 1% (w/v) chitosan solution degraded at 25 °C, regardless of the degree of N-acetylation. Lower values of $[\eta]_e$ are characteristic for 0.5% (w/v) chitosan solutions or for samples degraded at 4 °C. Thus, the decrease of temperature as well as concentration of chitosan solution results in decrease of $[\eta]_e$, which is equivalent in this study to the decrease in x_e. It was demonstrated by Mostafa (1958) that the value of x_e is independent of the initial chain length ($\bar{x}_0$), provided the intensity of ultrasound and experimental conditions are kept constant and degradation time is long enough. In the case of our investigation it means that values of $[\eta]_e$ in Table 1 may be related with F_A value of degraded chitosan, when all other factors affecting degradation are the same. This is really observed for system with chitosan solution of c equal 0.5% (w/v): $[\eta]_e$ decreases with decreasing F_A, what could mean that macromolecules with higher number of protonated -NH_3^+ groups are more susceptible for ultrasound action, and electrostatic repulsion between glucosamine units leading to expansion of the chitosan chain should play a role.

Table 1
Initial and limiting intrinsic viscosity ($[\eta]_0, [\eta]_e$) of degraded chitosans, and the general rate parameter value (k) for chitosans [0.28], [0.25] and [0.10] at varying temperature (T) and solution concentration (c)

F_A	$[\eta]_0$ (dl/g)	$c = 1.0\%$ (w/v)				$c = 0.5\%$ (w/v)	
		$T = 25$ °C		$T = 4$ °C		$T = 25$ °C	
		$[\eta]_e$ (dl/g)	$k \times 10^9$ (r^2) [mol/(L × h)]	$[\eta]_e$ (dl/g)	$k \times 10^9$ (r^2) [mol/(L × h)]	$[\eta]_e$ (dl/g)	$k \times 10^9$ (r^2) [mol/(L × h)]
0.28	12.9	5.5	8.38 (0.998)	5.1	15.0 (0.988)	5.0	9.13 (0.988)
0.25	13.2	4.8	4.82 (0.956)	4.4	11.3 (0.998)	4.4	5.53 (0.997)
0.10	15.0	5.1	2.49 (0.989)	4.4	8.17(0.999)	3.0	5.28 (1.000)

AcOH concentration, 0.1 M; $[\eta]$ value calculated as average of Matthes (1943); Solomon and Ciuta (1962) methods.

In the case of solutions with concentration of 1% (w/v), the trend described above is disturbed, what may be related to interactions between macromolecules in more concentrated solution. Papers dealing with this problem (Anthonsen, Vårum, Hermansson, Smidsrød, & Brant, 1994; Ottøy, Vårum, Christensen, & Anthonsen, 1996), showed that the nature of these interactions is complex.

Change of k with the value of the degree of N-acetylation always has the same direction: the lower value of F_A, the lower k is observed. This indication, however, should be discussed in detail since all experiments on polymer sonodegradation (Marx-Figini, 1997; Mostafa, 1958) showed the straight dependence of k on the value of $\bar{x}_0$. Values of k determined for chitosans, presented in Table 1, varying simultaneously with $\bar{x}_0$ and the degree of N-acetylation, reflect the influence of both these parameters. Separation of these effects was done by dividing k value by $[\eta]_0$, ($[\eta]_0$ is treated as a measure of x_0). Calculated values of k^+ ($k^+ = k/[\eta]_0$), independent on x_0, are depicted in Table 2.

Data in Table 2 show that the increase in the value of the degree of N-acetylation causes the strong increase of k^+ in the applied experimental conditions. The increase of k^+ is higher in the case of sonication of 1% (w/v) chitosan solution in 0.1 M AcOH at 25 °C ($k^+_{[0.28]}/k^+_{[0.10]} = 3.9$), and lower at 4 °C ($k^+_{[0.28]}/k^+_{[0.10]} = 2.5$). In other words, the increase of the k^+ value caused by the decrease of temperature is higher for more de-N-acetylated chitosans.

The direction of change of the k^+ values (Table 2) is the same as in the case of k (cf. Table 1): both decrease with decreasing F_A. Such dependence indicates stronger effect of F_A than of x_0 on the rate of sonochemical degradation of chitosan. This can be observed e.g. for samples [0.25] and [0.28] (differences in the initial viscosity average degree of polimerisation, $\bar{x}_{v,0}$, amounts 2% whereas in F_A it is 12%). The change of the general rate parameter, k, with F_A may be attributed to the influence of F_A on: (a) conformation of chitosan molecules, and on (b) degree of aggregation of chitosan chains, in solution of definite chitosan concentration.

3.2. Chitosan coil conformation

Chitosan chains of of the same x, but varying in the degree of N-acetylation have different conformations in dilute HOAc solution at a given concentration. Lower value of F_A, i.e. higher number of protonated amine groups, in solution of low ionic strength, may lead to the polyelectrolyte effect, so the macromolecule is more asymmetric, the chain is more expanded and should be more susceptible for sonication (Alexander & Fox, 1954; Basedow et al., 1978). However, the change of the degree of N-acetylation implies the change in molecular structure of chitosan molecules, which become more flexible when the degree of N-acetylation decreases. Thus, two opposite effects determine the conformation of chitosan molecules in solution. Studies performed by Anthonsen, Vårum and Smidsrød (1993) with chitosans of $0 \leq F_A \leq 0.6$ showed that there is a characteristic value of the number average degree of polymerisation ($\bar{x}_n$), above which the hydrodynamic volume of chitosan molecules is mostly determined by structural effects (the presence of bulky acetamide group making the backbone more rigid) and not by electrostatic interactions. This value of $\bar{x}_n$ was found to be dependent on ionic strength (μ): at $\mu \rightarrow 0$ it was equal ~ 800, and at $\mu \rightarrow 1$ mol/l it was equal ~ 300. In the case of our study, $\bar{x}_n$ measured by HPGPC system e.g. for chitosan[0.10] was determined to be ~ 1100, thus molecules of chitosan with higher F_A and in

Table 2
Values of k^+ (the general rate parameter of sonochemical degradation independent on degree of polymerization) for chitosans [0.28], [0.25] and [0.10] at varying temperature (T) and solution concentration (c)

$\bar{x}_{0,v}$	F_A	$k^+ \times 10^9$ [g × mol/(L^2 × h)]		
		$c = 1.0\%$ (w/v)		$c = 0.5\%$ (w/v)
		$T = 25$ °C	$T = 4$ °C	$T = 25$ °C
4179	0.28	6.50	13.7	7.08
4350	0.25	3.65	8.56	4.19
5406	0.10	1.66	5.45	3.52

AcOH concentration, 0.1 M; $\bar{x}_{0,v}$ – initial value of the average degree of polymerisation calculated from viscometric measurements.

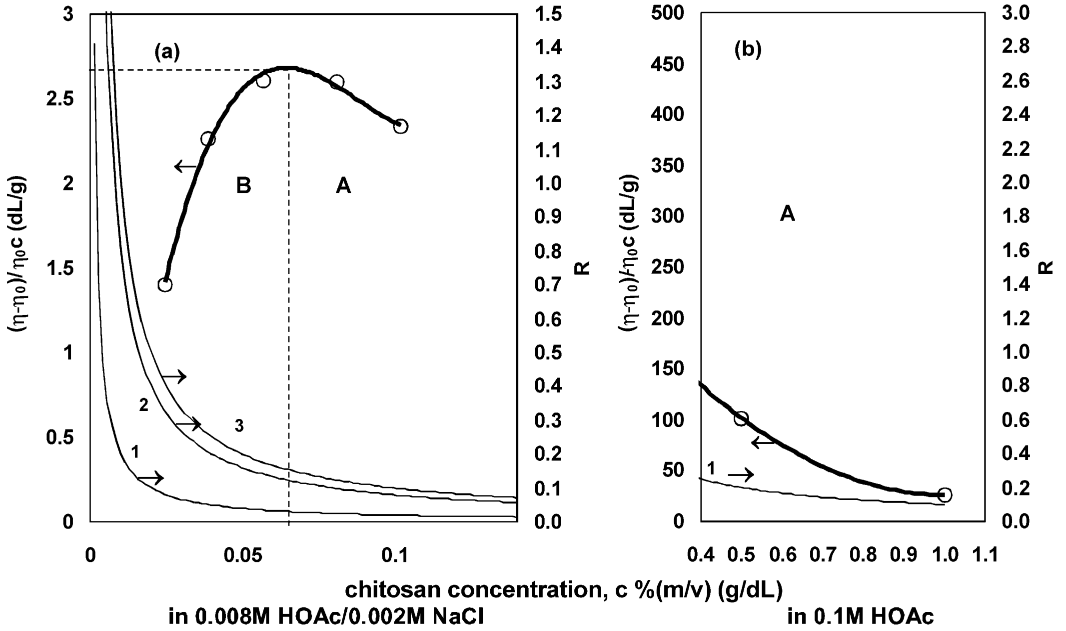

Fig. 2. Effect of electrostatic interactions on the hydrodynamic volume of chitosan as a function of solution concentration: increase (region A) and decrease (region B) of η_{sp}/c with dilution. Values (bold lines) of η_{sp}/c for chitosan [0.18] in solvent system: 0.008 M AcOH/0.002 M NaCl at concentration range 0.025–0.1%(m/v) with the maximum η_{sp}/c value (a) and for chitosan[0.28] in 0.1 M HOAc (b). Calculated R values (thin lines) are presented for AcOH (1), NaCl (2) and as a sum (3).

solution with low ionic strength were relatively more asymmetric (expanded) and the degradation by ultrasound could be more efficient (at least at earlier stages).

Since chitosan shows the polyelectrolyte effect in dilute acetic acid solutions, conformation of its molecules is also determined by the concentration of chitosan (in connection with the degree of *N*-acetylation) at low ionic strength. This behaviour is demonstrated in Fig. 2.

For low values of μ, characterised by the ratio (R) of the concentration of simple electrolyte (M) and concentration of chitosan, c (g/dl) (R is the measure of effective screening of charges along the chain, according to Kienzle-Sterzer, Rodrigues-Sanchez, & Rha (1982)), the increase of the reduced viscosity (η_{sp}/c) with decreasing c is observed (cf. Fig. 2, region A), namely the polyelectrolyte effect is indicated. As chitosan solution is being diluted, values of R increase and η_{sp}/c reaches its maximum (for $c = 0.0643$ g/dl and $R = 0.16$), what corresponds to maximum electrostatic interactions and maximum expansion of the macromolecular coil. Behind maximum, the increase of R with dilution becomes effective enough to screen the electrostatic charges and to suppress the polyelectrolyte effect (Fig. 2, region B). The described phenomenon is observed as well in dilute (Lyubina, Strelina, Nud'ga, Plisko, & Bogatova, 1983) as in semi-concentrated solutions (Rinaudo, Pavlov, & Desbrières, 1999). According to the criterion proposed by Bohdanecký (Bohdanecký & Kovař, 1982) concentrations of chitosan solution considered in this paper are situated in the semi-concentrated range ($1 < [\eta]_0 c \leq\sim 10$). The obtained values of η_{sp}/c for 0.5% (w/v) and 1% (w/v) chitosan solutions in 0.1 M HOAc (26 and 101 dl/g, respectively—Fig. 2b) indicate that expansion of chitosan chains with dilution takes place (in the considered range of chitosan concentration). This is consistent with the results presented by Rinaudo et al. (1999) for chitosan solutions in 0.1 M HOAc, where maximum expansion of chitosan chains is observed at $c \approx 0.2\%$ (m/v) and $R \approx 0.5$. This implies that more concentrated chitosan solutions used in this study [$c > 0.2\%$ (m/v)] are characterised by values of η_{sp}/c placed in the region of the decrease of η_{sp}/c with concentration, as it is shown in Fig. 2.

The above consideration indicates that the hydrodynamic volume of chitosan in 0.5% (w/v) solution is higher than that in 1% (w/v), for low value of R. As a consequence, the higher value of k in 0.5% (w/v) chitosan solution is due not only to lower solution concentration (what is usually observed), but additionally due to more assymetric (expanded) conformation of chitosan chains (more pronounced polyelectrolyte effect).

3.3. Aggregation of chitosan

As the decrease of chitosan concentration reduces its aggregation (Ottøy et al., 1996), the influence of aggregation on the value of the general rate parameter is lower, and in relatively dilute solution the effect of x_0 can be manifested. In Table 2 the results for chitosans [0.25] and [0.10], degraded at 25 °C, reveal such situation. In the case of 1% (w/v) chitosan concentration, the ratio $k^{+}_{[0.25]}/k^{+}_{[0.10]} = 2.2$, whereas for 0.5% (w/v) chitosan solution it is 1.2.

For a given value of the degree of *N*-acetylation (and x_0) value, k correlates with experimental conditions in the opposite way as does $[\eta]_e$: the decrease in temperature and/or concentration causes the increase of the general rate parameter of sonochemical degradation. This trend in dependence of $[\eta]_e$ and k upon experimental conditions is in agreement with papers reporting sonochemical action on other polymers (Thomas and Alexander, 1957) and also with the mechanism of sonication. The intensity of cavitation, which is the main reason of chain rupture, is more effective at lower temperature due to lower vapour pressure of the solvent and better solubility of gases. However, the decrease of temperature causes also the increase of chitosan solution viscosity, what means higher cohesive forces and lower effectiveness of cavitation. Therefore, the decrease of temperature introduces two features acting in opposite directions and the final value of k is a resultant of both these effects. The effect of polymer solution viscosity on cavitation is not large in many investigated systems (Basedow & Ebert, 1975). In the case of chitosan solution the temperature coefficient of intrinsic viscosity is negative [equal about -5.0×10^{-3} according to Trzciński, Vårum, Staszewska, Smidsrød and Bohdanecků (2002)] what means expansion of chitosan molecules with decreasing temperature and, as a consequence, the effect of solution viscosity is compensated, at least partly, by the increase of the intensity of cavitation.

Moreover, lower concentration of chitosan solution is accompanied with lower number of entanglements and lower solution viscosity, what facilitates the cavitation and makes degradation more effective. In the case of the system considered in this paper, the two-fold decrease of chitosan solution concentration caused approximately the same result on k as the decrease of temperature from 25 to 4 °C.

The influence of the concentration of HOAc in the used solvent on the rate of sonochemical degradation is shown in Fig. 1a, where the kinetic run for 1% (w/v) chitosan solution in 1 M HOAc is presented together with those for chitosan solutions in 0.1 M HOAc. For higher concentration of HOAc (1.0 M), the limiting intrinsic viscosity, $[\eta]_e$, is obtained after longer period of time (~1.2 h) in comparison with 0.1 M HOAc (~1.0 h), but its value is lower (3.04 dl/g) than in 0.1 M HOAc (4.37 dl/g) for the same chitosan sample [0.10]. This suggests that sonochemical degradation is accompanied additionally with chemical (acidic) degradation accelerated by ultrasound not only in strong acids, what was also shown in literature (Takahashi, 1997), but also in the relatively diluted (1.0 M) solution of acetic acid. The value of k for degradation carried out in 1% (w/v) chitosan solution in 1.0 M HOAc was found to be 6.0×10^{-9} mol/(L × h) ($r^2 = 0.989$), what is two-fold higher than that for the same chitosan sample in solution of 0.1 M HOAc (2.49×10^{-9} mol/(L × h)) ($r^2 = 0.989$). The obtained results are contrary to this obtained by Chen et al. (1997) for chitosan[0.30], however they confirm theoretical predictions (Basedow & Ebert, 1977) and investigations of Basedow and Ebert (1975) on dextrans sonolysis. These studies, (Basedow & Ebert, 1975, 1977) and suggestions of Doulagh (1978) showed that rate of sonochemical degradation depends upon solvating capacity of the solvent, as the energy released in cavitation process is more effective in good solvents, where the chains are relatively more extended than in poor solvents. On the basis of data reported by Rinaudo et al. (1999) (chitosan[0.12]), one can notice that the higher thermodynamic quality of the used solvent (1.0 M HOAc) can be the additional reason for higher k value of chitosan sonodegradation in 1.0 M HOAc.

3.4. Distribution of the degree of polymerisation

Investigations on time evolution of P of sonochemically degraded chitosan were carried out for samples [0.28], [0.23] and [0.18], characterised by the initial value of weight average degree of polymerisation ($\bar{x}_{w,0}$) equal 2042, 1094 and 1970, respectively. Distributions of x at degradation time of t ($t = 0$, 7, 15 and 120 min) are presented in Fig. 3. Additionally, effects of chitosan concentration and temperature on the x distribution pattern of degraded chitosans were analysed for sample [0.28].

As it is shown in Fig. 3, the bimodal shape of distribution of x is revealed after relatively short time of degradation, 7 and 15 min for chitosans [0.18] and [0.28] [$T = 25$ °C, $c = 1\%$ (w/v)], respectively (Fig. 3a and c). The gaussian character of the curve is reached after 120 min of degradation for sample [0.18] (Fig. 3c), whereas distribution of x of chitosan [0.28] remains bimodal after the same time of sonochemical degradation. Thus, the effect of the degree of *N*-acetylation on the time evolution of the polymerisation degree distribution curve of chitosans is evidently shown.

The bimodal distribution of x at early stages of sonochemical degradation of mono- and polydispersed polymers is related to the specific character of the process, namely to the preference of scission at the central segment of macromolecules (Basedow & Ebert, 1977; Mason & Lorimer, 1988). Results on sonochemical degradation of polystyrene (Glynn & van der Hoff, 1973; Smith & Temple, 1968), poly(*N*-vinylpyrrolidone) or pullulan (Koda, Mori, Matsumoto, & Nomura, 1994) confirm the non-random character of chain breakage. The distribution curves shown in this paper are well correlated with the specific character of sonochemicall degradation, especially when the mid-point breakage concept is considered. The analysis of the two shoulders revealed on the plot presented in Fig. 3a and c indicates that the value of the local maximum of the degree of polymerisation on the distribution curve for shoulder from the lower range of $x(x_{p,1})$ to that from the higher range of $x(x_{p,2})$ is 1/2. This clearly shows that chitosan molecules undergo not-random process of chain scission, with the most probable site of breakage at the mid-point of the chain. Numerical values are collected in Table 3. The observed mechanism of breakage results in the initial increase of the P_w value, which is expected to decrease in further stages of degradation [or even to oscillate when $\bar{x}_0$ is the multiplication of x_e, what was shown i.e. by Smith and Temple

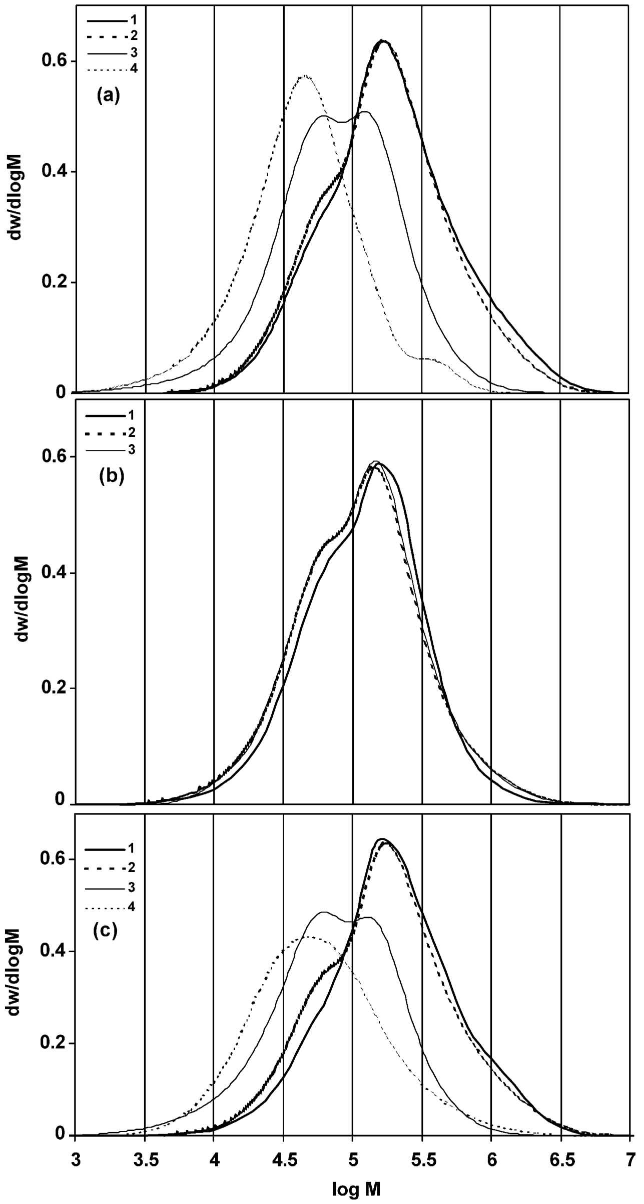

Fig. 3. Distribution curves of molecular weight of mother and sonochemically degraded chitosans; (a) [0.28]: (1), non degraded; (2), $c = 1\%$ (w/v), $t = 15$ min, (3), $c = 1\%$ (w/v), $t = 120$ min, (4), $c = 0.05\%$ (w/v), $t = 7$ min; (b) [0.23], $c = 1\%$ (w/v): (1), non degraded, (2), $t = 7$ min, (3), $t = 15$ min, and (c) [0.18], $c = 1\%$ (w/v): (1), non degraded, (2), $t = 7$ min, (3), $t = 15$ min, (4), $t = 120$ min. ($T = 25$ °C)

Table 3
Values of $x_{p,1}$ and $x_{p,2}$ (local maximum of the degree of polymerisation after 120 and 15 min of sonochemical degradation, respectively, cf. Fig. 3a and c) and their ratios for chitosans [0.28] and [0.18]

Sample	$\log x_{p,1}$	$\log x_{p,2}$	$x_{p,1}$	$x_{p,2}$	$x_{p,1}/x_{p,2}$
[0.28]	2.526	2.825	335	670	0.50
[0.18]	2.534	2.838	345	690	0.50

$T = 25$ °C, $c = 1\%$ (w/v).

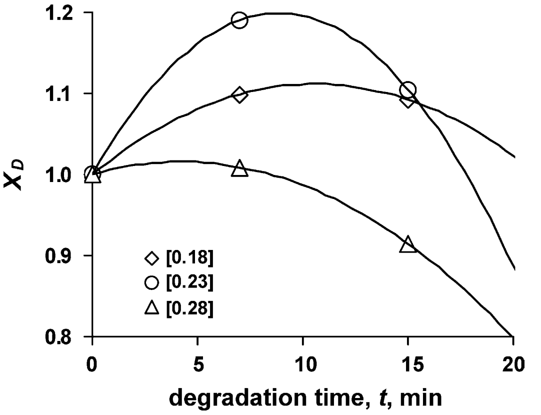

Fig. 4. Changes of P_w of sonochemically degraded chitosans [0.28], [0.23] and [0.10] during the course of degradation in normalized scale: $X_D = P_{w,t}/P_{w,0}$.

(1968)]. The transitional increase of the P_w value was found to be the highest for the sample with the lowest $P_{w,0}$ value, i.e. chitosan [0.23]. These changes of P_w value in the course of degradation are summarised in Fig. 4 in normalised scale.

The influence of aggregates on time evolution of the x distribution curve is manifested by relatively slower decrease of x in the case of chitosan [0.28] at the earliest stages of sonochemical degradation, possibly because of disruption of more aggregated chains (higher value of F_A) in comparison to chitosan [0.18]. This trend is also observed in the case of chitosan[0.23], though changes in distribution of x are relatively small. Such behaviour might be caused by lower value of $\bar{x}_{w,0}$ (1094) of sample [0.23] in comparison to samples [0.28] and [0.18] (2042 and 1970, respectively).

The additional changes of distribution of x due to decrease of temperature are not remarkable, though the rate of sonochemical degradation is higher at lower temperature (cf. Table 1.). The shape of the distribution curve of chitosan[0.28] degraded at 5 °C (data not shown) [$t = 7$ min, $c = 1\%$ (w/v)] is comparable to that presented for chitosan[0.28] degraded at 25 °C (other experimental conditions the same) (Fig. 3a). Only slightly higher susceptibility of longer macromolecules for sonication is observed at 5 °C. As it was stated in Section 2, the decrease of temperature causes the increase of solution viscosity [negative temperature coefficient of [η], according to Trzciński et al. (2002)] parallel with the decrease of vapour pressure of the solvent components and better solubility of gases. The action of these effects on sonochemical efficiency cancel each other and as ΔT is relatively small ($\Delta T = 20$ °C), the influence of temperature on k is diminished. Considerable differences are detected in the case of sonication of diluted [0.05% (w/v)] solution of chitosan [0.28]. As it was shown (Fig. 3a), relatively strong decrease of average values of x and monomodal distribution of x are indicated after 7 min of degradation. This observation is in agreement with mechanism of cavitation, which is more effective in low-viscous and diluted solutions of macromolecular compounds. Regularities

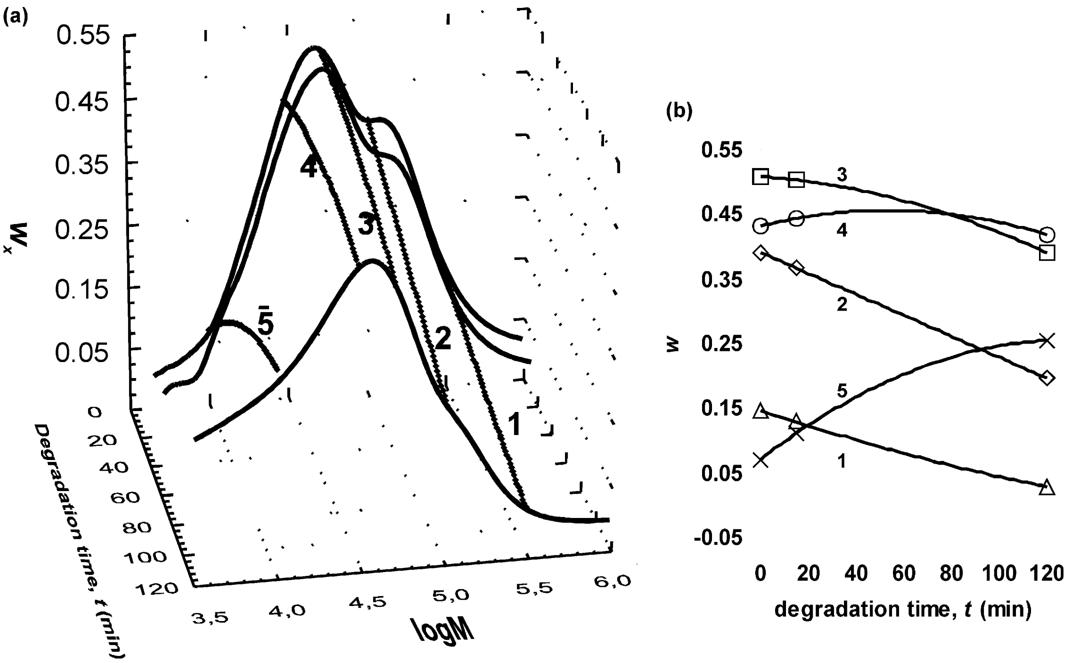

Fig. 5. Three dimensional diagram of distribution of molecular weight of chitosan[0.28] as a function of time (a) and time evolution of x for $x = 1730$ (1), 580 (2), 290 (3), 170 (4) and 60 (5) (b).

between sonodegradation rate constants and concentration of the polymer solution indicates the increase of rate of degradation with decreasing concentration or maximum rate of sonochemical degradation in the range of so-called critical concentration, c^* (Bohdanecký & Kovař, 1982). This value is characteristic for a given polymer-solvent system and defines the minimal concentration of the polymer solution when entanglements occur. Value of c^* may be estimated on the basis of the weight average squared radius of gyration, $(\bar{R}_G^2)_w^{1/2}$, following the formula:

$$c^* = \frac{3\bar{x}_w M_0}{4\pi N_A (\bar{R}_G^2)_w^{3/2}} \tag{4}$$

where N_A is the number of Avogadro. For chitosan [0.28] $[\bar{x}_w = 2042, M_0 = 173.0\ \mathrm{g/mol}$ and $(\bar{R}_G^2)_w^{1/2} = 54.6\ \mathrm{nm}]$[1] calculated c^* is 8.55×10^{-2} g/dl, what explains the observed strong increase of degradation rate for the diluted solution of chitosan [0.28].

Comparing the initial distribution of x of original chitosan [0.28] to distribution of x after degradation time of $t \neq 0$ for the same sample, both the value of x and the concentration (expressed in term of weight fraction, w_x) of fractions with definite x are altered during degradation. These changes may be summarised in the one three-dimensional $x - w_x - t$ diagram shown in Fig. 5a. The variation in w_x of chitosan[0.28] molecules with definite x is presented by planes parallel to the $w_x - t$ plane, which intercepts the x axis (Fig. 5b). It is clearly shown that curves $w_x - t$ are different for the different x. In the range of high values of x, only degradation takes place and the w_x of these molecules decreases monotonously with time. However, the rate of this decrease is not a straight dependence of x, and the maximum decrease of w_x during sonochemical degradation relates to molecules with $x \sim 600$, indicating the fastest degradation for this value of x. At small and medium values of x, the degradation of larger molecules causes the monotonic or intermediate increase of w_x, what is determined by the balance between molecules that are degraded and those formed due to degradation of molecules with relatively high value of x. This diagram is thus the schematic representation of the kinetic degradation models that are mostly applied to describe the sonochemical degradation of polymers (Glynn et al., 1972; Kotliar, 1964a,b). Later, continuous-distribution kinetics are used for data presented in this paper.

3.5. Application of continuous-distribution kinetic model

Continuous-distribution kinetics is a straight-forward technique which, taking into account the time evolution of P allow to determine the degradation rate coefficients. Time dependence of distribution of the degree of polymerisation is thus possible to express as $P = p(x, t)$, and $p(x, t)\mathrm{d}x$ is the molar concentration of the polymer of $x \in (x, x + \mathrm{d}x)$. Detail description of the procedure applied in the continuous-distribution kinetics can be found in literature (Madras et al., 2000; McCoy & Madras, 1997). One of the main parameter used in this model is the number of scission per chain (r), which can be estimated from experimental data. Usually, r is equal 2 or 3 for degradations performed in mild conditions (McCoy & Madras, 1997). From calculations it is

---

[1] Calculated on the basis of the dependence $R_G = f(\bar{x}_w)$ presented by Rinaudo, et al. (1993).

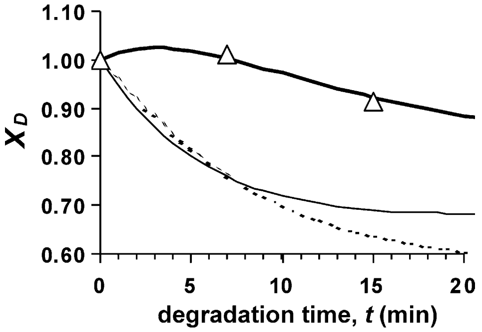

Fig. 6. Continuous-distribution kinetics model prediction of degradation time dependence of $X_D = X_n/X_w$ (bold line) for chitosan [0.28] end experimental values (Δ) (cf. Fig. 4). Additionally calculated curves of $X_n = \bar{x}_{n,t}/\bar{x}_{n,0}$ and $X_w = \bar{x}_{w,t}/\bar{x}_{w,0}$ for number of scission per chain $r = 3$ are presented (normal and dashed line, respectively). Rate coefficients: $k_1 = 1.1 \times 10^{-1}\ \text{min}^{-1}$, $k_2 = 2.9 \times 10^{-2}\ \text{min}^{-1}$ and $k_3 = 1.2 \times 10^{-2}\ \text{min}^{-1}$.

possible to obtain values of X_D (normalised change of P_w) and rate coefficients (k) for each scission.

Fig. 6 relates the experimental values of X_D to model predictions for $r = 3$ ($k_1 = 0.085$, $k_2 = 0.0265$ and $k_3 = 0.0115\ \text{min}^{-1}$, from regression analysis). The differences between values of k is in good correlation with the general rule of dependence of rate of sonolysis with $\bar{x}_0$, however these values are the average for chains differing in length ($P_{w,0} = 3.4$). For polydisperse samples, i.e. when the $P_{w,0}$ value is > 1.5, the decrease of X_D below 1 (and P_w below its initial value) is also observed in many experimental systems and the model (fitted for mid-point scission) also predicts this behaviour in our system, if k's are not of the same value.

3.6. Conclusion remarks

The results presented in this paper showed that sonolysis of chitosans is the process of specific mechanism, which lead to remarkable decrease of polymerisation degree to the level determined by the degree of *N*-acetylation and the applied experimental conditions.

Appropriate selection of the original material and parameters of the sonolysis allow the production of low molecular weight (not chitooligomers) and low polydispersive chitosans.

References

Alexander, P., & Fox, M. (1954). The role of free radicals in the degradation of high polymers by ultrasonic and by high-speed stirring. *Journal of Polymer Science*, *12*, 533–541.

Andrady, A. L., Torikai, A., & Kobatake, T. (1996). Spectral sensitivity of chitosan photodegradation. *Journal of Applied Polymer Science*, *62*, 1465–1471.

Anthonsen, M. W., Vårum, K. M., Hermansson, A. M., Smidsrød, O., & Brant, D. A. (1994). Aggregates in acidic solutions of chitosans detected by static laser light scattering. *Carbohydrate Polymers*, *25*, 13–23.

Anthonsen, W. A., Vårum, K. M., & Smidsrød, O. (1993). Solution properties of chitosans: conformation and chain stiffness of chitosans with different degrees of *N*-acetylation. *Carbohydrate Polymers*, *22*, 193–201.

Basedow, A. M., & Ebert, K. H. (1975). Zum Mechanismus des Abbaus von Polymeren in Lösung durch Ultraschall. *Die Makromolekulare Chemie*, *176*, 745–757. (in German).

Basedow, A. M., & Ebert, K. H. (1977). Ultrasonic degradation of polymers in solution. *Advances in Polymer Science*, *22*, 84–148.

Basedow, A. M., Ebert, K. H., & Foβhag, E. (1978). Ultrasonic degradation of Polymers in mixed solvents. *Die Makromolekulare Chemie*, *179*, 2565–2568.

Bohdanecký, M., & Kovař, J. (1982). *Viscosity of Polymer Solutions*. Amsterdam: Elsevier, pp. 166–167.

Chen, R. H., Chang, J. R., & Shyur, J. S. (1997). Effects of ultrasonic conditions and storage in acidic solutions on changes in molecular weight and polydispersity of treated chitosan. *Carbohydrate Research*, *299*, 287–294.

Doulagh, M. S. (1978). A proposed mechanism for the degradation of addition polymers in cavitating ultrasonic fields. *Journal of Applied Polymer Science*, *22*, 1735–1743.

Folkman, W., & Pospieszny, H. (2001). Molecular basis of systemic aquired resistanced induced by chitosan and its oligomers. In H. Struszczyk (Ed.), progress on Chemistry and Application of Chitin and it's derivatives, Łódz (Poland): Polish Chitin Society.

Glynn, P. A. R., & van der Hoff, B. M. E. (1973). Degradation of polystyrene in solution by ultrasonation. A molecular weight distribution study. *Journal of Macromolecular Science-Chemistry*, *A7*, 1695–1719.

Glynn, P. A. R., van der Hoff, B. M. E., & Reilly, P. M. (1972). A general model for prediction of molecular weight distribution of degraded polymers. *Journal of Macromolecular Science—Chemistry*, *A6*, 1653–1664.

Jellinek, H. H. G., & White, G. (1951). The degradation of long-chain molecules by ultrasonic waves. IV. The dependence of rate constant on concentration for polystyrene. *Journal of Polymer Science*, *7*(1), 33–37.

Kienzle-Sterzer, C. A., Rodrigues-Sanchez, D., & Rha, C. K. (1982). Dilute solution behavior of a cationic polyelectrolyte. *Journal of Applied Polymer Science*, *27*, 4467–4470.

Koda, S., Mori, H., Matsumoto, K., & Nomura, H. (1994). Ultrasonic degradation of water-soluble polymers. *Polymer*, *35*, 30–33.

Kotliar, A. M. (1964a). A critical evaluation of mathemetical molecular weight distribution models proposed for real polymer distributions. I. Effects of a low molecular weight cut-off value. *Journal of Polymer Science: Part A*, *2*, 4303–4325.

Kotliar, A. M. (1964b). A critical evaluation of mathemetical molecular weight distribution models proposed for real polymer distributions. II. Effects of a high molecular weight cut-off value. *Journal of Polymer Science: Part A*, *2*, 4327–4337.

Lorimer, J. P., Mason, T. J., Cuthbert, T. C., & Brookfield, E. A. (1995). Effect of ultrasound on the degradation of aqueous native dextran. *Ultrasonic Sonochemistry*, *2*, S55–S57.

Lyubina, S. Ya., Strelina, I. L., Nud'ga, L. A., Plisko, Ye. A., & Bogatova, I. N. (1983). Flow birefringence and viscosity of chitozane solutions in acetic acid at various ionic power. *Vysokomolarnye Soedinenia*, *A25*, 1467–1472. (in Russian).

Madras, G., Kumar, S., & Chattopadhyay, S. (2000). Continuous distribution kinetics for ultrasonic degradation of polymers. *Polymer Degradation and Stability*, *69*, 73–78.

Marx-Figini, M. (1997). Studies on the ultrasonic degradation of cellulose. *Die Angewandte Makromolekulare Chemie*, *250*, 85–92.

Mason, T. J., & Lorimer, J. P. (1988). *Sonochemistry: theory, applications and uses of ultrasound in chemistry*. Chichester: Ellis Horwood Ltd, pp. 99–138.

Matthes, A. (1943). The viscometric determinations of the degree of polymerization and configurational relations of polyamides in solution. *Zeitschrift für praktische Chemie*, *162*, 245–278. (in German) (C.A. 1944,38,2546^8).

McCoy, B. J., & Madras, G. (1997). Degradation kinetics of polymers in solution: dynamics of molecular weight distributions. *American Institute of Chemical Engineering Journal (AIChEJ)*, *43*, 802–810.

Mostafa, M. A. K. (1958). Degradation of addition polymers by ultrasonic waves. V. Effect of initial average chain length. *Journal of Polymer Science*, *33*, 295–310.

Muzzarelli, R. A. A., & Rocchetti, R. (1985). Determination of the degree of acetylation of chitosans by first derivative ultraviolet spectrophotometry. *Carbohydrate Polymers*, *5*, 461–472.

Ottøy, M. H., Vårum, K. M., Christensen, B. E., & Anthonsen, M. W. (1996). Preparative and analytical size-exclusion chromatography of chitosans. *Carbohydrate Polymers*, *31*, 253–261.

Pantaleone, D., Yalpani, M., & Scollar, M. (1992). Unusual susceptibility of chitosan to enzymic hydrolysis. *Carbohydrate Research*, *237*, 325–332.

Portenlänger, G., & Heusinger, H. (1997). The influence of frequency on the mechanical and radical effects for the ultrasonic degradation of dextranes. *Ultrasonic Sonochemistry*, *4*, 127–130.

Ratajska, M., Struszczyk, M. H., Boryniec, S., Peter, M. G., & Loth, F. (1997). The degree of deacetylation of chitosan: optimisation of the IR method. *Polimery (Warsaw)*, *42*, 572–575.

Rathke, T. D., & Hudson, S. M. (1994). Review of chitin and chitosan as fiber and film formers. *Journal of Macromolecular Science—Reviews of Macromolecular Chemistry and Physics*, *C34*, 375–437.

Rinaudo, M., Milas, M., & Dung, P. L. (1993). Characterization of chitosan. Influence of ionic strength and degree of acetylation on chain expansion. *International Journal of Biological Macromolecules*, *15*, 281–285.

Rinaudo, M., Pavlov, G., & Desbrières, J. (1999). Influence of acetic acid concentration on the solubilization of chitosan. *Polymer*, *40*, 7029–7032.

Roberts, G. A. F. (1992a). *Chitin Chemistry*, Houndmills and London: McMillan Press Ltd, pp. 1–53.

Roberts, G. A. F. (1992b). *Chitin Chemistry*, London: McMillan Press Ltd, pp. 254–256.

Roberts, G. A. F. (1997). Chitosan production routes and their role in determining the structure and properties of the product. In A. Domard, G. A. F. Roberts, & K. M. Vårum (Eds.), *Advances in Chitin Science* (*vol. 2*) (pp. 22–31). Lyon: André.

Roberts, G. A. F., & Domszy, J. G. (1982). Determination of the viscometric constants for chitosan. *International Journal of Biological Macromolecules*, *4*, 374–377.

Schmid, G. (1940). Zur Kinetik der Ultraschalldepolymerisation. *Zeitschrift der Physikalische Chemie*, *186*, 113–128. (in German).

Smith, W. B., & Temple, H. W. (1968). Polymer studies by gel permeation chromatography. IV. The degradation of polystyrene by ultrasonics and by benzoyl peroxide. *The Journal of Physical Chemistry*, *72*(13), 4613–4619.

Solomon, O. F., & Ciută, I. Z. (1962). Détermination de la Viscositè Intrinsèque de Solutions de Polymères par une Simple Détermination de la Viscosité. *Journal of Applied Polymer Science*, *6*, 683–686.

Suzuki, S., Suzuki, K., Tokoro, A., Okawa, Y., & Suzuki, M. (1986). Immunopotentiating effect of *N*-acetyl-chito-oligosaccharides. In R. A. A. Muzzarelli, C. Jeuniaux, & G. W. Gooday (Eds.), *Chitin in Nature and Technology* (pp. 485–492). *Proceedings of the Third International Conference on Chitin and Chitosan, Senigallia, Italy*, London: Plenum Press.

Takahashi, Y. (1997). Effect of sonication on the acid degradation of chitin and chitosan. In A. Domard, G.A.F. Roberts, & K.M. Vårum (Eds.), *Advances in Chitin Science*, *vol. 2*, pp. 372–377.

Tan, S. C., Khor, E., Tan, T. K., & Wong, S. M. (1998). The degree of deacetylation of chitosan: advocating the first derivative UV-spectrophotometry method of determination. *Talanta*, *45*, 713–719.

Thomas, B. B., & Alexander, W. J. (1957). Ultrasonic degradation of cellulose nitrate. II. Effects of temperature, solvent, and other process variables. *Journal of Polymer Science*, *25*, 285–304.

Trzciński, S., Vårum, K. M., Staszewska, D. U., Smidsrød, O., & Bohdanecký, M. (2002). Comparative studies on molecular chain parameters of chitosans and poly(diallyldimethylammonium chloride): the stiffness B-parameter and the temperature coefficient of intrinsic viscosity. *Carbohydrate Polymers*, *48*, 171–178.

Ulański, P., & Rosiak, J. M. (1992). Preliminary studies on radiation induced changes in chitosan. *International Journal of Radiation Applications and Instrumentation. Part C. Radiation Physics and Chemistry*, *C39*, 53–57.

Ulański, P., Wojtasz-Pająk, A., Rosiak, J. M., & von Sonntag, C. (2000). Radiolysis and sonolysis of chitosan—two convenient techniques for a controlled reduction of the molecular weight. In M. G. Peter, A. Domard, & R. A. A. Muzzarelli (Eds.), (*vol. 4*) (pp. 429–435). *Advances in Chitin Science*, Schmergow, Germany: Druckhaus.

Vårum, K. M., & Ottøy, M. H. (2001). Acid hydrolysis of chitosans. *Carbohydrate Polymers*, *46*, 89–98.