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Effects of ultrasonic conditions and storage in acidic solutions on changes in molecular weight and polydispersity of treated chitosan

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Abstract

The objective of this study is to explore the effects of ultrasonic conditions and storage in acidic solution on changes in the molecular weight and polydispersity (molecular weight distribution) of treated chitosan. The ultrasonic conditions studied include parameters of chitosan concentration, reaction temperature, type of solvent, and ultrasonic time. The results show that chitosan was degraded faster in dilute solutions and faster in lower temperature solutions. Degradation increased with prolonged ultrasonic time, and chitosan was degraded during storage in an acidic solution at ambient temperatures. The polydispersity decreased with ultrasonic treatment for all ultrasonic conditions studied and during storage in acidic solution. Polydispersity decreased from 10.10 to 2.11, 3.11, 4.04, and 5.09 for 0.2%, 0.8%, 1.4%, and 2.0% solutions, respectively. Polydispersity decreased to 4.04 and 4.15 for solutions treated at 4 °C and 50 °C, respectively. Polydispersity decreased to 4.04 and 4.31 for chitosan treated in acetic acid buffer and 5% acetic acid, respectively. Polydispersity decreased to 2.64 after prolonged (120 min) ultrasonic treatment. After storage in acetic acid buffer at ambient temperatures for 17 days, polydispersity changed from 10.10 to 6.86 for untreated and from 6.02 to 3.62, from 3.02 to 2.86, and from 2.64 to 2.34 for those chitosans subjected to ultrasonic degradation treatment for 10 min, 60 min, and 120 min, respectively.

Keywords: Chitosan; Ultrasonics; Molecular weight; Polydispersity

1. Introduction

Chitinous material is the second-most abundant biopolymer [1], and is considered to be a versatile, environmentally friendly raw material [2]. It can be used in many areas, such as food processing, biochemistry, pharmaceuticals, medicine, and agriculture [3–5]. Functional properties such as viscosity [6], antimicrobial activity [7], immunoadjuvant activity [8], hypercholesterolemic activity [9], mechanical properties and porosity of membranes [10–12], blood coagulation activity, and wound-healing activity [13] of chitinous materials depend on the molecular weight

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and degree of deacetylation (dd) of the product used. The molecular weight and dd of obtained chitosans depend on the conditions of preparation. Alkali treatment at elevated temperatures is a very common, effective method used to produce various molecular weight and dd chitinous materials. However, the alkali depolymerization procedure creates environmental problems. An energy-saving, environmentally friendly, and effective method has been proposed [14,15]. Sonication [16,17], γ-irradiation [18], and other methods can be used to degrade the $(1 \rightarrow 4)$-β-linkage (depolymerization) and effect the deacetylation of chitinous materials. The principle behind the above physical means is to provide the added energy needed to break the chemical bonds. Studies on the effect of ultrasonic conditions on the degradation of polymers are numerous. Lorimer et al. [19] studied the effect of ultrasonic intensity, reaction temperature, and solution concentration on the degradation rate constant of dextran. Basedow et al. [20], and Basedow and Ebert [21] reported that solvent characteristics have a considerable effect on the degradation rate. Ohta et al. [22] reported that the degradation rate constant decreased linearly with decreasing molecular weight. Apparent rate constants decreased gradually and approached zero when molecular weights equaled 7×10^3 Da. Mathematical models for degradation of polymers by ultrasonic radiation are numerous. Koda et al. [23] considered that the ultrasonic degradation process should be represented by a multi-step reaction. However, with a short sonication time, the reaction may be assumed to be a first-order reaction. Malhotra [24] reported that poly(alkyl methacrylate) was randomly degradated by ultrasonic radiation. Koda et al. [23] reported that polydispersity is a useful quantity which can shed light on the degradation mechanism. Casale and Porter [25] reported that polydispersity depends on the reaction mechanism viz a specific versus random rupture process along the chain. Polydispersities have values of 2 for the case of random scission; 1–2 for rupture near mid-chain; and close to 2 if a random step is superimposed on an initial non-random scission after the extensive reaction. However, the effects of ultrasonic conditions on changes of molecular weight and dd of treated chitosan have rarely been studied, and the results are sometimes controversial. Wang and Lin [16] reported that ultrasonic treatment decreases the molecular weight while maintaining the dd of the treated chitosan. However, Muzzarelli and Rocchetti [17] reported that sonification leads to an immediate chain degradation and to detectable deacetylation after more prolonged periods of treatment, especially at a pH of 1.0. Effects of ultrasonic treatment on changes of dd of treated chitosan in these two reports do not agree with each other.

This study explores the effects of ultrasonic conditions and holding in acidic solution on changes in molecular weight and polydispersity (molecular weight distribution) of treated chitosan. Ultrasonic conditions studied were chitosan concentration, reaction temperature, type of solvent, and ultrasonic time.

2. Materials and methods

Chitosan preparation.—Chitin was prepared from shrimp (Solenocera prominentis) waste by the modified method of Stanley et al. [26] Ground shrimp waste was treated with 0.5 N NaOH at ambient temperatures to hydrolyze the surface flesh. The alkali-treated waste was washed, then dried and disintegrated to obtain powder. The powder was passed through sieves of 40 to 60 mesh. The flake-free powder was soaked in 2 N HCl for 2 h to remove the minerals until CO_2 evolution ceased. The demineralized powder was soaked in 2 N NaOH at 80 °C to hydrolyze the protein, and then it was washed with water until neutral. The alkali-treated power was soaked in 1% $KMnO_4$ at room temperature for 1 h to oxidize the astaxanthin, then soaked in 1% oxalic acid at 60 °C for 1 h to neutralize the $KMnO_4$. The product was then washed and dried to get a white chitin powder. Chitin powder was alkali treated (50% NaOH) at 100 °C for 2 h to get 70% dd chitosan. This was washed and dried at 50 °C to obtain the final products.

Determination of the degree of deacetylation.—The colloid titration method of Toei and Kohara [27] was followed. An aliquot of 0.50 g of chitosan was dissolved in 99.50 g 5% (v/v) acetic acid. One gram of chitosan-acetic acid solution was well mixed with 30 mL of deionized water. After adding 2 to 3 drops of the indicator, 0.1% toluidine blue, the solution was titrated with N/400 PVSK (potassium polyvinylsulfate, $(C_2H_3O_4SK)_n$, $n = 1500$ or above). The PVSK solution was calibrated with a cetylpyridinium chloride monohydrate standard solution (ca 0.8 g/L). Cetyl pyridinium was recrystallized from acetone and dried in a desiccator. The degree of deacetylation was calculated with the following equations:

$$\text{Degree of deacetylation} = \left[\frac{x/161}{x/161 + y/203}\right] * 100,$$

$x = \frac{1}{400} * \frac{1}{1000} * f * 161 * v$

(to calculate the weight of glucosamine),

$y = 0.5 * \frac{1}{100} - x$

(to derive the weight of N-acetyl glucosamine),

v: milliliters of N/400 PVSK used in titration,

f: factor of N/400 PVSK solution.

Molecular weight determination by the SE–HPLC method (size-exclusion high-performance liquid chromatography).—The method of Yomota et al. [28] was followed. A column (7.8 mm × 30 cm) packed with TSK gel G5000 PW_{XL} (Tosoh, Japan) was used. The mobile phase consisted of 0.2 M HOAc–0.1 M NaOAc, and 0.008 M NaN_3. Sample concentration of 0.1% (w/v) was loaded and eluted with a flow rate of 0.5 mL/min by an LDC Analytical ConstaMetric 3500 pump. The elute peak was detected by an RI detector (Gilson, model M132, USA). The data was analyzed by Chem-Lab software (Scientific Information Service Corporation, Taiwan). Chitosans with known molecular weights (determined by light scattering method) were used as references. The standard curve of elution volume and molecular weight was established. The weight-average molecular weights of the samples were calculated from the standard curve with the Chem-Lab software.

Calculation of polydispersity (molecular weight distribution).—Polydispersity (molecular weight distribution) of untreated and ultrasonic degraded chitosans were calculated from the ratio of M_w/M_n.

Here $M_w = \Sigma w_i M_i / \Sigma w_i$ and $M_n = \Sigma w_i / \Sigma[w_i/M_i]$; w is the weight of species i of molecular weight M_i and can be obtained from SE–HPLC chromatography. Polydispersity was calculated by Chem-Lab software.

Ultrasonic treatment.—Solutions of 2.0%, 1.4%, 0.8%, and 0.2% (w/v) chitosan–0.2 M HOAc–0.1 M NaOAc, pH 4.4, were prepared. The solutions were passed through filter paper (55 mm, # 1 Toyo Roshi Kaisha Ltd., Japan) to remove insoluble materials. A 75-mL aliquot of the filtrate in a glass vessel was placed in a water bath at a pre-set temperature. Ultrasonic treatments were conducted at 300 W for 2, 5, 10, 30, 60, 90, and 120 min with a sonicator (VCX 600, Sonic and Materials Inc., USA) with a 1/2″ extender (630–0410). An aliquot of the sample was pippetted out to analyze its molecular weight by SE–HPLC. The remaining solution was dialyzed to remove salts with a dialysis bag (Spectra/Por MwCo 3500, Spectrum, USA). The residues were collected and lyophilized.

3. Results

Effect of chitosan concentration.—The effect of ultrasonic conditions on the changes of molecular weight of treated chitosan was expressed as the molecular weight decrease ratio (MWDR). The MWDR was calculated as follows:

$MWDR = 1 - M_t/M_0$.

Here M_t and M_0 are the molecular weights of treated chitosan at time t and untreated chitosan, e.g., at time zero, respectively. Larger values of MWDR indicate greater decreases in molecular weight after ultrasonic treatment or greater molecular degradation occurring after ultrasonic treatment.

Fig. 1a shows that the MWDR of ultrasonic (300 W) treated chitosans increased rapidly during the first 10 min of treatment, then slowed during the remaining treatment time for all four concentrations studied at 4 °C. MWDRs were 0.75, 0.56, 0.37, and 0.42 for 0.2%, 0.8%, 1.4%, and 2.0% chitosan solutions, respectively, for the initial 10-min treatment. The MWDRs were near 0.79, 0.69, 0.56 and 0.50 for the

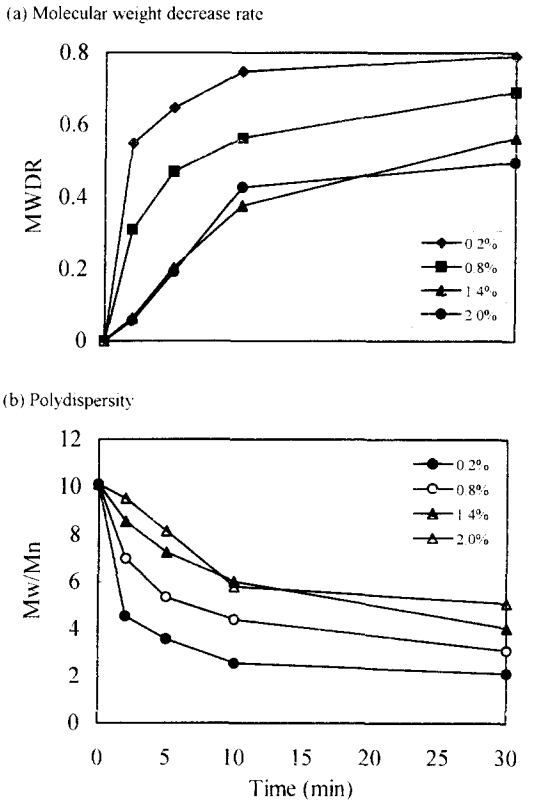

Fig. 1. Effect of chitosan concentration (0.2%, 0.8%, 1.4%, and 2.0%) in 0.2 M HOAc–0.1 M NaOAc on (a) the molecular weight decrease rate, and (b) polydispersity during ultrasonic treatment (300 W) at 4 °C.

solutions mentioned, respectively, at the end of treatment. The MWDRs increased faster for dilute solutions than for concentrated solutions. Fig. 1b shows that the polydispersity of chitosan at time zero was 10.10 and were 2.11, 3.11, 4.04, and 5.09 for 0.2%, 0.8%, 1.4%, and 2.0% chitosan solutions, respectively, at the end of treatment.

Effect of reaction temperature.—Fig. 2a shows the effect of reaction temperature on changes of MWDR of 1.4% chitosan in 0.2 M HOAc–0.1 M NaOAc solution during ultrasonic treatment (300 W). The MWDR increased to 0.42 and 0.33 for chitosan treated at 4 °C and 50 °C, respectively, for the initial 10 min of treatment. The MWDR increased at a slower rate during the remaining treatment time and reached 0.61 and 0.54 for chitosan treated at 4 °C and 50 °C, respectively, at the end of treatment. The MWDR of chitosan treated at 4 °C was 4% to 8% higher than that treated at 50 °C. Fig. 2b shows that the polydispersity of 1.4% chitosan in 0.2 M HOAc–0.1 M NaOAc solution was 10.10 at the beginning and 4.04 and 4.65 for chitosan treated at 4 °C and 50 °C, respectively, at the end of treatment.

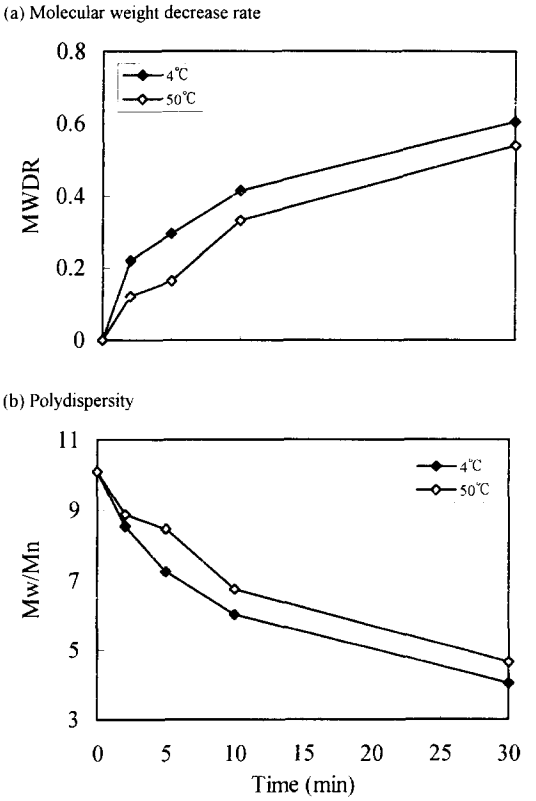

Fig. 2. Effect of ultrasonic (300 W) temperature (4 °C vs 50 °C) on (a) the molecular weight decrease rate, and (b) polydispersity of 1.4% chitosan in 0.2 HOAc–0.1 M NaOAc solution during ultrasonic treatment.

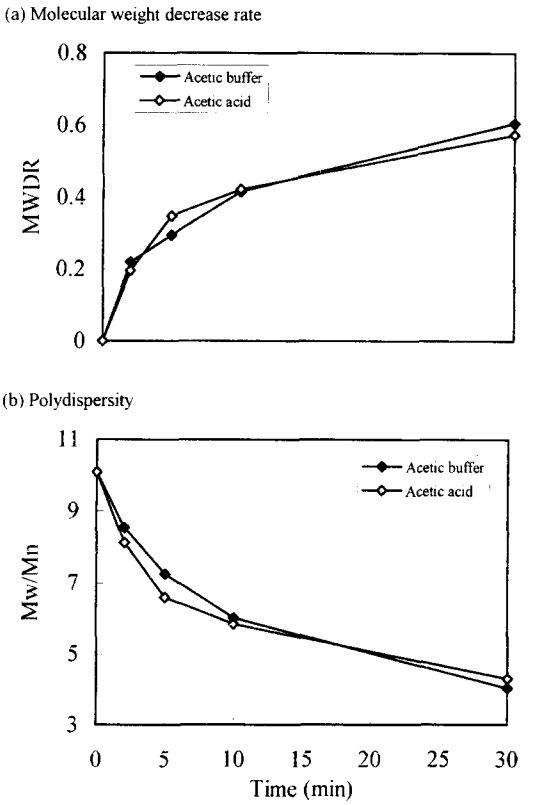

Fig. 3. Effect of different solvents (5% HOAc vs 0.2 M HOAc–0.1 M NaOAc) on (a) the molecular weight decrease rate, and (b) polydispersity of a 1.4% chitosan solution during ultrasonic treatment (300 W) at 4 °C.

Effect of the type of solvent.—Fig. 3a shows that the MWDR of 1.4% chitosan in 5% HoAc, pH 2.6, was similar to that in 0.2 M HOAc–0.1 M NaOAc, pH 4.4, during the time course of treatment (300 W, 4 °C). The MWDRs of treated chitosans in both solutions increased to 0.42 and 0.57 after the initial 10 min of treatment and at the end of treatment, respectively. Fig. 3b shows that the polydispersities of 1.4% chitosans in both type of solvents were 10.10 at time zero and were 4.04 and 4.31 in 0.2 M HOAc–0.1 M NaOAc and in 5% HoAc, respectively, at the end of treatment.

Effect of ultrasonic time.—Fig. 4a shows the effect of ultrasonic (300 W) time on the changes of MWDR of 1.4% chitosan in 0.2 M HOAc–0.1 M NaOAc solution at 4 °C. The MWDR increased very quickly during the initial 15 min of treatment, then slowed gradually and approached an asymptote at about 100 min. Fig. 4b shows that polydispersity of 1.4% chitosan in 0.2 M HOAc–0.1 M NaOAc was 10.10 at the beginning and were 3.02, 2.82, and 2.64

after 60 min, 90 min, and 120 min of treatments, respectively.

Effect of holding in acidic solution.—Fig. 5a shows the effect of holding a 1.4% (w/v) chitosan (treated for 10, 60, and 120 min, and an untreated control) in 0.2 M HOAc–0.1 M NaOAc solution at ambient temperatures for 17 days on the changes of its MWDR. The molecular weights of untreated chitosan, and those treated for 10, 60, and 120 min were 1.85×10^6, 7.93×10^5, 2.34×10^5, and 1.39×10^5 Da, respectively, before the start of the holding experiment. The increase in the MWDR of untreated chitosan was larger than those subjected to ultrasonic treatment for various times during holding in 0.2 M HOAc–0.1 M NaOAc. The changes in MWDRs of those ultrasonic-treated chitosans did not differ significantly. Fig. 5b shows that the polydispersity of 1.4% untreated chitosan and those of 10 min treatment, of 60 min treatment, and of 120 min treatment decreased from 10.10 to 6.06, from 6.02 to 3.02, from 3.02 to 2.86, and from 2.64 to 2.34, respectively, after storage in 0.2 M HOAc–0.1 M NaOAc at ambient temperatures for 17 days.

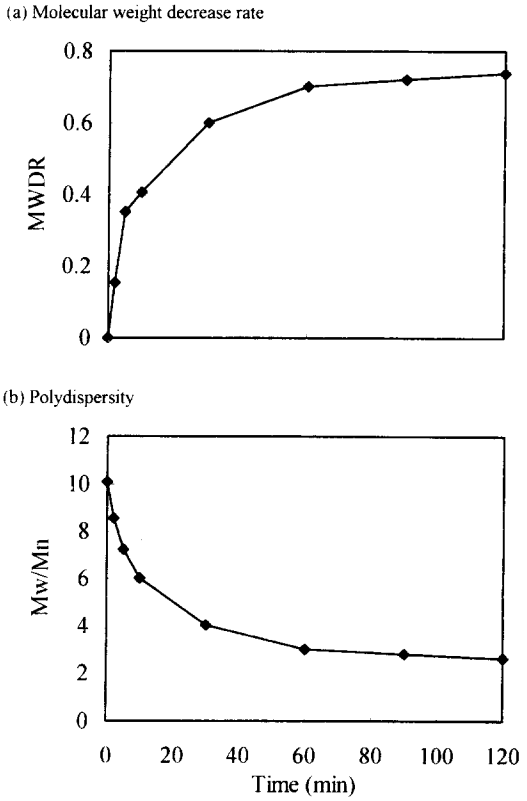

Fig. 4. Effect of ultrasonic time (300 W) on (a) the molecular weight decrease rate, and (b) polydispersity of 1.4% chitosan in 0.2 M HOAc–0.1 M NaOAc solution at 4 °C.

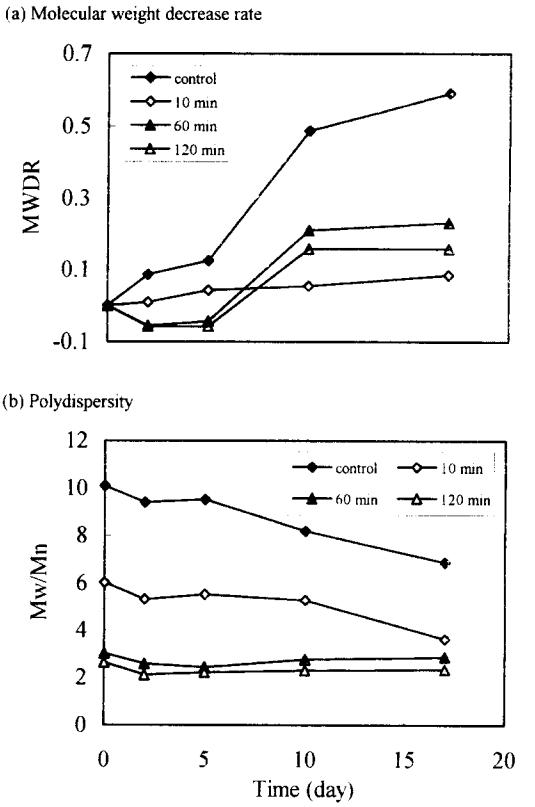

Fig. 5. Effect of keeping in 0.2 M HOAc–0.1 M NaOAc solution of control or ultrasonic-treated samples (300 W) at room temperature for various times on (a) the molecular weight decrease rate, and (b) polydispersity of 1.4% chitosan at 4 °C.

4. Discussion

Effect of chitosan concentration.—Results in Fig. 1a show that the higher the chitosan concentration, the slower the resulting increase in the MWDR during ultrasonic treatment, which indicates the concentrated solution alleviated the depolymerization reaction. The results are consistent with those concerning the degradation of polystyrene and dextran in solution by ultrasonic treatment reported by Glynn et al. [29] and Lorimer et al. [19], respectively. Lorimer et al. [19], reported that for a given temperature, the degradation rate appeared to be concentration dependent. The degradation rate decreased with increasing concentration, which may be because, at the same level of energy input, the energy needed to degrade the polymer was diluted by the increase of chitosan concentration. Fig. 1b shows that the polydispersity

of chitosan at time zero was 10.10 and were 2.11, 3.11, 4.04, and 5.09 for 0.2%, 0.8%, 1.4%, and 2.0% chitosan solutions, respectively, at the end of treatment. Thus before ultrasonic treatment, the chitosan sample has a broader molecular weight distribution. After 30 min of treatment, the molecular weight distribution becomes smaller as revealed by the decrease in polydispersity. Decrease in polydispersity was faster and larger for dilute solutions than for concentrated solutions. This indicates that larger molecular weight chitosans degrade faster in dilute solutions and result in a narrower molecular weight distribution. Casale and Porter [25] reported that the molecular weight distribution in terms of M_w/M_n depends on the reaction mechanism, e.g, a specific versus a random rupture process along the chain after extensive reaction. Polydispersity has values of 2 representing random scission degradation; values of 1–2 for the case of rupture near midchain; and values close to 2 if a random step is superimposed on an initial nonrandom scission. The polydispersity of 2.11 of 0.2% chitosan reported herein is close to 2. This indicates that after 30 min of treatment, the molecular weight distribution is near the most probable distribution for a random scission process after extensive degradation [25], and it implies that ultrasonic treatment for 30 min is enough time for a 0.2% chitosan solution to be extensively degraded. However, polydispersities of 3.11, 4.04, and 5.09 for 0.8%, 1.4%, and 2.0% solutions, respectively, are far from the 2.0 value, indicating that a treatment time of 30 min is insufficient to get extensively degraded products for those solutions. This reaffirms that the degradation rate decreases with increasing concentration. Based on polydispersity values of 2.11, and degradation models of Casale and Porter [25] and Koda et al. [23], ultrasonic degradation of chitosan may represent by a random step being superimposed on an initial nonrandom scission.

Effect of reaction temperature.—The results in Fig. 2a show that the higher the reaction temperature, the lower the resulting increase in MWDR during ultrasonic treatment, which indicates that elevating the solution temperature is detrimental to the degradation process by ultrasonics. Lorimer et al. [19] reported similar results. The degradation rate of dextran at concentration between 2% and 10% increased by decreasing the reaction temperature. However, Chen et al. [30] reported the MWDR of 2% chitosan increased to 0.3 by just storing the solution at 80 °C for 2 h, and the MWDR of 1.4% chitosan sheared (26,000 s^{-1}) at 4 °C was 5% to 27% lower than that sheared at 40 °C for 30 min. Both results indicate that high reaction temperatures facilitate the degradation process. The results of ultrasonic treatment at higher reaction temperatures are inconsistent with the results of shearing or just keeping the solution at elevated temperatures. This may be due to the cavitation bubble generated by the ultrasonic wave more easily escaping out of solution from higher temperature solutions than from lower temperature solutions [22]. The shearing effect as the cavitation bubble collapses is considered to be the major force degrading the polymer during ultrasonic treatment [23]. Therefore, elevating solution temperatures during ultrasonic treatment will retard the depolymerization effect. Fig. 2b shows that at the end of treatment, the polydispersity had decreased from 10.10 to 4.04 and 4.65 for solutions treated at 4 °C and 50 °C, respectively. The decreases in dispersity of chitosans treated at 4 °C were larger than those treated at 50 °C, which indicates that chitosan was degraded faster at 4 °C than at 50 °C and resulted in a narrower molecular weight distribution. However, polydispersities of 4.04 and 4.65 indicate that the treated chitosan had not been completely degraded even after 30 min of treatment.

Effect of solvent.—Fig. 3a shows that the changes of the MWDR of treated chitosan in 5% HOAc were similar to that in 0.2 M HOAc–0.1 M NaOAc. The conformation of chitosan in 5% HOAc solution should be more extended than that in 0.2 M HOAc–0.1 M NaOAc [31]. This indicates that the effect of the conformation of chitosan on the degradation rate by ultrasonic treatment was insignificant during the time course of treatment. Basedow and Ebert [21] reported that solvent characteristics have a considerable effect on the degradation rate. Degradation rates increased with increasing degree of expansion of the polymer in solution. Chen et al. [30] that reported the increase of MWDR of 1.4% chitosan in 5% HoAc was 1% to 15% higher than that in 0.2 M HOAc–0.1 M NaOAc when sheared (26,000 s^{-1}) at 4 °C for 30 min. These results are consistent with those of Basedow and Ebert [21]. The changes in the MWDR of treated chitosan in those two solvents treated by ultrasonics are not in accord with results of Basedow et al. [20] and Basedow and Ebert [21] during the time course of treatment. These inconsistences require further studies. Fig. 3b shows that after 30 min of treatment, the polydispersity of chitosan in 0.2 M HOAc–0.1 M NaOAc and in 5% HoAc did not differ significantly and were 4.04 and 4.31, respectively. Results in Fig. 3a and Fig. 3b show that the type of solvent did not significantly affect the degradation rate of chitosan

by ultrasonic treatment, and the treated chitosan was not extensively degraded after 30 min of treatment.

Effect of treatment time.—The results in Fig. 4a indicate that ultrasonic degradation on the 1.4% chitosan solution is most effective during the first 15 min of treatment. The degradation rate approached an asymptote during the remainder of the treatment time. The limited degradation rate which occurred in the long run resulted in narrowing the molecular weight distribution of the degraded chitosan. This may be due to degradation rates decreasing with decreasing molecular weight [19,22]. These results are consistent with the report of Wang et al. [32]. Ohta et al. [22] reported that the degradation rate constant of dextran by ultrasonic treatment decreased gradually and approached zero at a molecular weight of 7×10^3 Da, and that there exists a limiting value of molecular weight after exhausting treatment. However, Basedow and Ebert [33] reported that when dextran was ultrasonified at 82 °C in 0.6 M phosphoric acid, the rate constant is proportional to the molecular weight increase to the power of 4/3 when the molecular weight is higher than the limiting value. However, the rate constant is proportional to the molecular weight raised to the power of 5/6 for molecular weights below the limiting value. The results in Fig. 4a suggest that the degradation rate approaches zero as the molecular weight decreases to 1.8×10^5 Da. Results in Fig. 4b show that the polydispersity is 2.64 after 120 of min treatment. A polydispersity of 2.64 is close to 2.0, the most probable distribution for the random scission process, after exhausted degradation which implies that perhaps 120 min is enough time of ultrasonic treatment for a 1.4% chitosan solution to be extensively degraded.

Effect of holding in acidic solution.—Results in Fig. 5a indicate that acid hydrolysis [34,35] occurred at ambient temperatures when holding chitosan in 0.2 M HOAc–0.1 M NaOAc solution for a long period of time. The extent of acid hydrolysis was remarkable for high molecular-weight chitosans, but less extensive for lower molecular-weight chitosans. However, Anthonsen et al. [36] and Matsumoto et al. [37] reported that chitosan aggregates in acidic solutions as observed by light scattering, although, the aggregation is concentration dependent. Results in Fig. 5a indicate degradation occurred especially for the control or for chitosan treated for 10 min. The fact that no aggregates were detected in those samples may be due to the aggregates being concentration dependent and the concentration of aggregates being too low to be detected by SE–HPLC, or may be attributed to different sensitivities in detecting aggregates by SE–HPLC and by light scattering. The results also indicate that the ultrasonic-degraded products did not aggregate to form higher molecular-weight products during holding in 0.2 M HOAc–0.1 M NaOAc solution for a long period of time. Results in Fig. 5b that show the polydispersities of 1.4% untreated chitosan and those of 10 min treatment, of 60 min treatment, and of 120 min treatment decreased from 10.10 to 6.06, from 6.02 to 3.02, from 3.02 to 2.86, and from 2.64 to 2.34, respectively, after keeping in 0.2 M HOAc–0.1 M NaOAc at ambient temperatures for 17 days. This indicates that polydispersities become narrower for untreated chitosan and for chitosan after 10 min of treatment. However, there are no significant differences in chitosans treated for 60 min and 120 min. The results indicate that acid hydrolysis occurred preferentially on those chitosans whose structures were subjected to no or slight ultrasonic degradation.

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