Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 340 (2005) 2403–2410
Carbohydrate RESEARCH

The enzymatic degradation and swelling properties of chitosan matrices with different degrees of *N*-acetylation

Dongwen Ren,[a,b] Hongfu Yi,[a,b] Wei Wang[a] and Xiaojun Ma[a,*]

[a]*Laboratory of Biomedical Material Engineering, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian 116023, China*
[b]*Graduate School of the Chinese Academy of Sciences, 19 Yuquan Road, Beijing 100039, China*

Received 19 April 2005; received in revised form 11 July 2005; accepted 12 July 2005
Available online 18 August 2005

Abstract—In the design of chitosan-based drug delivery systems and implantable scaffolds, the biodegradation rate of the chitosan matrix represents a promising strategy for drug delivery and the function of carriers. In this study, we have investigated the degradation of chitosan with different degrees of *N*-acetylation, with respect to weight loss, water absorption, swelling behavior, molecular weight loss of bulk materials, and reducing sugar content in the media. Chitosan matrices were prepared by compression molding. The results revealed that the initial degradation rate, equilibrium water absorption, and swelling degree increased with decreasing degree of deacetylation (DD) and a dramatic rise began as DD of the chitosan matrix decreased to 62.4%. Chitosan matrices with DD of 52.6%, 56.1%, and 62.4% had the weight half-life of 9.8, 27.3, and more than 56 days, respectively, and the weight half-life of average molecular weight 8.4, 8.8, and 20.0 days, respectively. For chitosan matrices with DD of 71.7%, 81.7%, and 93.5%, both types of half-life exceeded 84 days because of the much slower degradation rate. The dimension of chitosan matrices during degradation was determined by the process of swelling and degradation. These findings may help to design chitosan-based biomedical materials with predetermined degradation timed from several days to months and proper swelling behaviors.

Keywords: Enzymatic degradation; Chitosan matrices; *N*-Acetylation; Biomedical materials

1. Introduction

Chitosan, a copolymer of D-glucosamine and *N*-acetyl-D-glucosamine linked through β-(1→4) glycosidic linkages, is a partially deacetylated derivative of chitin, the primary structural polymer of arthropod exoskeletons.[1] The term chitosan usually refers to a family of polymers including not only those derived from chitin by *N*-deacetylation,[2] but also those derived from chitosan by *N*-acetylation,[3] including the block and random copolymer structures thus obtained.[4] The molecular weight and degree of *N*-deacetylation (DD) are thought to be the two most important determinants of the properties of chitosan.[5] Chitosan exhibits good biocompatibility and biodegradability,[6–8] and has been widely explored for applications in controlled drug delivery and tissue engineering.[9–15]

The degradation behavior of chitosan plays a crucial role on the long-term performance of a tissue-engineered cell/material construct. The degradation kinetics may affect many cellular processes, including cell growth, tissue regeneration, and host response.[16] Chitosan has been shown to be degraded mainly by lysozyme (EC 3.2.1.17),[17] which commonly exists in various human body fluids and tissues with concentrations from 4 to 13 mg/L[18] in serum and from 450 to 1230 mg/L in tears.[19] Many investigations have been published on the degradation of chitosan by lysozyme.[6–8,20–22] All showed that the DD of chitosan is one of the key factors controlling the degradation of chitosan. Among them, Aiba studied and compared the lysozymic digestibility of chitosans with block-type and random-type copolymer structures in acetate buffer at pH 5.4 by measuring

* Corresponding author. Tel.: +86 411 84379139; fax: +86 411 84379096; e-mail: maxj@dicp.ac.cn

0008-6215/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2005.07.022

the reducing sugar content and the molecular weight of the hydrolysates in solution.[20] Recently, Freier also showed that degradation of chitosan could be controlled by *N*-acetylation based upon measurements of the weight loss of chitosan films in lysozyme solution.[23] In addition, the swelling property is another important factor in drug delivery and tissue engineering, such as in protein release,[24] artificial cartilage,[25] and nerve regeneration.[26] Aiba found that the swelling of chitosan films depended on the degree of acetylation of original chitosan.[4]

However, until now most studies have focused on the lysozymic degradation of chitosan in acidic solution by measuring the viscosity and molecular weight changes,[8,20–22] or in chitosan films by measuring the weight loss.[6,7] Few reports have been published on how chitosan bulk materials behave during the lysozymic degradation process, such as changes in molecular weight or swelling properties. Such details are very important to applications of chitosan-based biomedical materials.

The aim of this paper is to comprehensively investigate the degradation and swelling behaviors of chitosans with different degrees of *N*-acetylation during the lysozymic degradation process. These studies were done in PBS buffer at pH 7.4 and degradation was monitored by following weight loss, swelling, water absorption, and changes in molecular weight. A better understanding of this process will improve our ability to control functional properties of chitosan to fulfill their promise as biomaterials.

2. Experimental

2.1. *N*-Acetylation of chitosan and matrices fabrication

Chitosan with a DD of 93.5% was supplied by Yuhuan Ocean Biomaterials Corporation (Yuhuan, China). The weight average molecular weight (M_w) was 83,600 with a polydispersity index (PI) of 1.78 as determined by gel permeation chromatography. The polydispersity index (PI) is equal to the ratio of weight average to number average molecular weight (M_w/M_n).

N-Acetylation of chitosan was performed according to the method of Hirano et al.[3] Briefly, 2 g chitosan powder was dissolved in 50 mL of 2% (v/v) acetic acid solution. After a period of time, the solution was filtered through 0.45 μm membrane and 50 mL MeOH was added to produce a transparent yellowish solution. Additional Ac_2O was then added and the solution was kept at room temperature overnight and then neutralized with 1 mol/L NaOH, which produced a white precipitate. The precipitate was washed with double distilled water several times until neutral and then freeze-dried for 24 h. Chitosans with various degrees of *N*-acetylation were obtained by controlling the amount of Ac_2O added. The products were passed through a 200-mesh sieve and kept in a dry container before use.

FTIR spectra of chitosan samples were recorded as KBr pellets on a Vector 22 spectrometer (Bruker, Germany). Samples and KBr were fully dried prior to submission of samples by FTIR analyses to exclude influence of water. The DD of chitosan samples was calculated from Eq. 1:

$$\mathrm{DD} = (1 - 1.15 \times A_{1655}/A_{3450}) \times 100\% \quad (1)$$

where A_{1655} and A_{3450} are the absorbance intensity at 1655 cm^{-1} and 3450 cm^{-1}, respectively.[27]

X-ray powder diffraction patterns of the chitosan samples were measured on a Rigaku D/max-rC diffractometer at 40 kV and 25 mA with a radiation of λ 1.5418 Å. The relative intensities were recorded within the range of 5–40° (2θ) at a scanning rate of 2°/min. The crystallinity index (CrI) and d-space in the direction of (1 1 0) crystal plane were calculated as previously reported.[28]

Chitosan matrices were fabricated by compression molding using a laboratory press. In brief, 100 mg of chitosan powder was transferred into a cylindrical hardened steel mold (13 mm id) and then compressed under 10 MPa of pressure for 5 min to yield matrices of 13 mm in diameter and about 0.7 mm in thickness.

2.2. In vitro enzymatic degradation

The initial matrices were placed in 50 mL vials containing 25 mL of 0.1 mol/L, pH 7.4, phosphate-buffered saline (PBS) containing 0.5 mg/mL lysozyme (70,000 U/mg, Fluka, USA) and 0.5 mg/mL NaN_3. The vials were stored in an incubator (Haerbin Donglian, China) set at 37 °C and agitated at 120 rpm. Three samples of each matrix type were collected at fixed time intervals and rinsed thoroughly with doubly distilled water to remove any buffer and lysozyme remaining on the surface, and blotted with filter paper to remove surface water. Individual samples were measured to obtain wet weights $W_{t,h}$, thickness H_t, and diameter D_t. Samples were dried at room temperature and then freeze-dried for 24 h, and weighed to obtain dry weights $W_{t,d}$ and kept in a dry container for further measurements. The supernatant in each vial was withdrawn to measure the content of reducing sugar.

Water absorption was calculated from Eq. 2:

$$\%\ \mathrm{H_2O} = 100 \times (W_{t,h} - W_{t,d})/W_{t,d} \quad (2)$$

where equilibrium water was expressed as a percentage of dry weight.

The remaining weight of the chitosan matrices was calculated after the enzymatic degradation using Eq. 3:

$$\%\ \text{Weight remaining} = 100 \times W_{t,d}/W_0 \quad (3)$$

The half-life of weight was defined as the time when $W_{t,d}$ equals $W_0/2$.

The swelling degree was calculated from Eq. 4:

$$\% \text{ Swelling} = 100 \times (D_t/D_0)^2 \times (H_t/H_0) \quad (4)$$

2.3. Molecular weight determination by gel permeation chromatography (GPC)

The molecular weights of the initial chitosan films and degraded samples were measured by GPC equipped with a refractive index detector (Waters, Model 2414, Milford, Massachusetts, USA) and an HPLC pump (Waters, Model 515). The samples were dissolved in an acetate buffer of 0.2 mol/L CH_3COOH/0.1 mol/L CH_3COONa and eluted through a TSK G4000PWxl column (7.8 × 300 mm, 10 μm particle diameter, Tosoh Corporation, Tokyo, Japan) with a 20 μL injection volume at a flow rate of 0.6 mL/min. The column and the detector were both set at 30 °C. Pullulan standards (Shodex Standard P-82, Showa Denko K.K., Japan) were used for a calibration curve. The half-live of the M_w was defined as the time when the M_w equaled half of molecular weight of the original sample.

2.4. Reducing sugar determination

The content of reducing sugar in the supernatant was estimated by the modified Schales method.[29] Briefly, after a given time of degradation, 1.5 mL of supernatant was taken in a test tube and mixed with 2 mL of potassium ferricyanide solution prepared by dissolving 0.5 g of potassium ferricyanide in 0.5 mol/L Na_2CO_3 to 1 L. The tube was sealed with aluminum foil and kept in a water bath at 100 °C for 15 min, then cooled to room temperature within 5 min. The absorbance of the solution was measured at 420 nm in a UV–vis spectrophotometer (Shimadzu 2550, Japan). The values of the reducing sugar could be read from the calibration curve of D-glucosamine.

3. Results and discussion

3.1. *N*-Acetylation of chitosan and initial matrices

Chitosan is composed of *N*-acetyl-D-glucosamine and D-glucosamine and can be regarded as a copolymer of the two units. The distribution of acetyl groups along the polysaccharide chain is considered to influence its properties. *N*-Acetylation of highly deacetylated chitosan under homogeneous conditions gave random-type copolymers of the two units.[4] Using this method, six chitosan samples were obtained by adding various amounts of acetic anhydride. Their DDs were 93.5%, 81.7%, 71.7%, 62.4%, 56.1%, and 52.6% as determined from FTIR spectra (Fig. 1) with Eq. 1 and were marked as Chi93, Chi81, Chi71, Chi62, Chi56, and Chi52, respectively. FTIR spectra revealed that the intensity of amide band I at 1650 cm^{-1} increased when more acetic anhydride was added, which clearly indicates *N*-acetylation of the amino groups in the chitosan molecule. This method was found to be reproducible and the influence of the ratio of acetic anhydride to chitosan on the resulting DD is presented in Figure 2. It was found that *N*-acetylation was difficult when the DD of the chitosan fell below 60%. This might be ascribed to the crystallinity changes of chitosan materials upon *N*-acetylation. X-ray diffraction patterns in Figure 3 showed that the peak at 20° (2θ) became weaker from Chi93 to Chi56. Similarly, CrI (Table 1) also showed this decreasing tendency, which suggests that the introduction of the acetyl groups breaks the crystal zone in chitosan making it less ordered in structure. However, enough acetyl groups also gave another crystal form at 10° (2θ), one type of hydrated crystal named Form I according to Samuels,[30] and thus the degree of order of chitosan molecular structure increased (CrI 59.5% in Table 1).

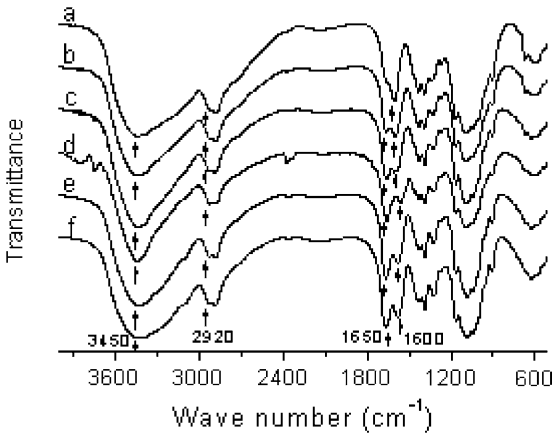

Figure 1. FTIR spectra of chitosans: (a) Chi93; (b) Chi81; (c) Chi71; (d) Chi62; (e) Chi56; (f) Chi52.

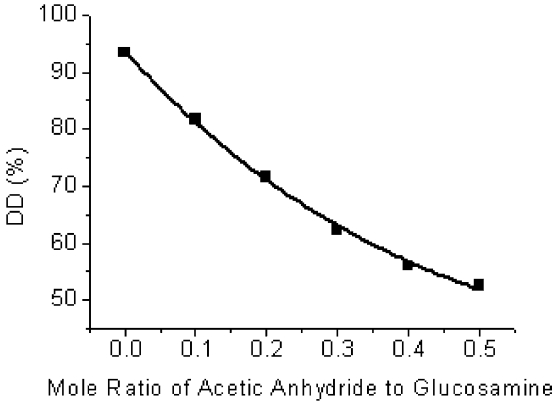

Figure 2. DD of chitosans as a function of the mole ratio of acetic anhydride to glucosamine units during *N*-acetylation.

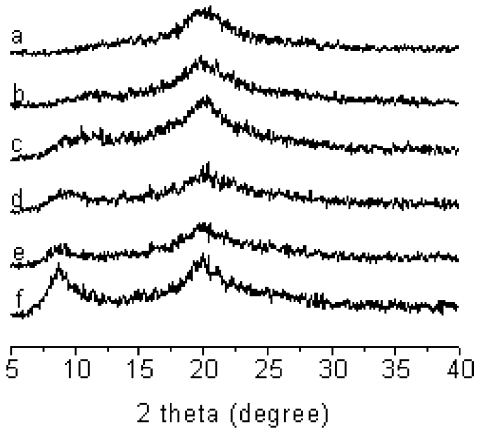

Figure 3. X-ray diffraction patterns of chitosans with different DD: (a) Chi93; (b) Chi81; (c) Chi71; (d) Chi62; (e) Chi56; (f) Chi52.

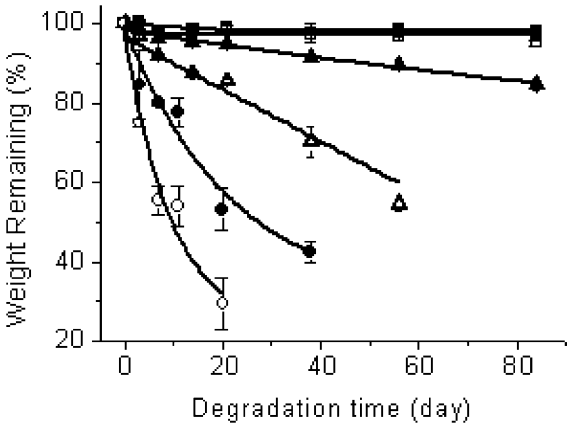

Figure 4. Change in weight remaining of chitosan matrices undergoing in vitro degradation by lysozyme: (■) Chi93; (□) Chi81; (▲) Chi71; (△) Chi62; (●) Chi56; (○) Chi52.

Table 1. The influence of *N*-acetylation to the crystal properties of chitosan

Sample code	DD (%)	CrI (%)	2θ (°)	d-space (Å)
Chi93	93.5	62.2	20.6	4.31
Chi81	81.7	54.3	11.8, 19.9	7.50, 4.46
Chi71	71.7	52.0	10.6, 20.4	8.35, 4.35
Chi62	62.4	47.0	9.6, 20.5	9.21, 4.33
Chi56	56.1	44.3	9.3, 20.2	9.51, 4.40
Chi52	52.6	59.5	8.8, 20.1	10.05, 4.42

Table 2. Properties of fabricated chitosan matrices

Matrices code	Diameter (mm)	Weight (mg)	Thickness (μm)	DD (%)	Mw (Da)	PI
Chi93	13	90.3 ± 0.6	636 ± 48	93.5	83 600	1.78
Chi81	13	90.5 ± 0.3	650 ± 38	81.7	97 270	1.85
Chi71	13	92.5 ± 0.7	707 ± 98	71.7	98 530	1.85
Chi62	13	92.1 ± 1.4	662 ± 34	62.4	98 500	1.84
Chi56	13	92.1 ± 0.9	662 ± 41	56.1	100 280	1.69
Chi52	13	92.1 ± 1.1	660 ± 43	52.6	102 870	1.63

Six chitosan matrices were fabricated by compression molding with chitosan powder (Table 2). The matrices had a diameter of 13 mm and a thickness from 636 to 707 μm. The weight of the matrices was recorded after freeze-drying for 24 h and these ranged from 90.3 to 92.1 mg. All fabricated chitosan matrices had similar M_w (~90 000) and PI (~1.7).

3.2. Weight, water absorption, and swelling

The weight of chitosan matrices Chi93, Chi81 remained relatively constant throughout degradation (Fig. 4). The weight of matrix Chi71 decreased slightly and 85% of the original weight still remained after 84 days (Fig. 4). In contrast, the other three matrices, Chi62, Chi56, and Chi52, all experienced dramatic weight loss with 85%, 60%, and 30% remaining after 21 days, respectively. At the end of the experiments, 56 days for Chi62, 38 days for Chi56, and 21 days for Chi52, only 55%, 45%, and 30% weight remained, respectively, indicating their much faster degradation (Fig. 4). At the end of this period, the three types of matrices were much thinner and more fragile, and no samples could be retrieved. These results verified the influence of the DD on the degradation of chitosan, as reported.[6–8,20–23] However, previously only such a profile was given with no further details. To elucidate the influence of the DD, the initial slopes of the curves in Figure 4 were plotted as the initial degradation rate against the DD in Figure 5. An obvious increase of the initial degradation rate appeared when the DD of chitosan decreased to 62.4%. The initial degradation rate was only about 0.2%/day for Chi93, Chi81, and Chi71, and increased to 0.8%/day, 3.0%/day, and 7.8%/day for Chi62, Chi56, and Chi52, respectively. These results were consistent with a recent study by Tomihata and Ikada.[7] They investigated the degradation of chitin and its deacetylated

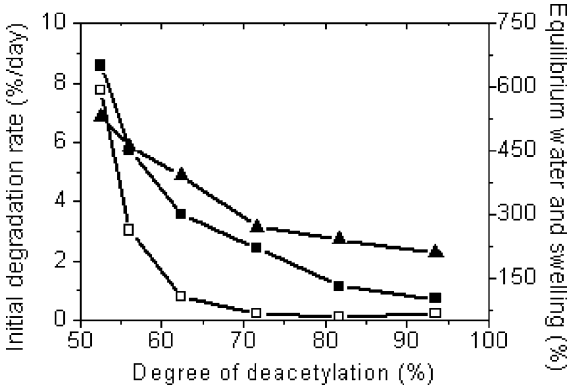

Figure 5. Dependence of initial degradation rate (□), equilibrium water absorption (■), and equilibrium swelling degree (▲) of chitosan matrices as a function of the DD. The initial degradation rate is calculated from the initial slopes of the curves in Figure 4.

derivatives in the form of films and found that the in vivo biodegradation rate experienced a dramatic increase when the DD decreased to 73.3%. According to Aiba,[20] chitosans derived from chitin by *N*-deacetylation in NaOH solution, which have a block-type copolymer structure, has a faster degradation rate at an early stage, while chitosan, with random-type copolymer structure degraded less quickly, such as Chi62 in this study, although they had similar DD.

The weight half-lives of chitosan matrices were obtained from Figure 4 and are listed in Table 3. Matrices Chi52, Chi56, and Chi62 had half-lives of 9.8, 27.3, and more than 56 days, respectively, while the half-lives of Chi71, Chi81, and Chi93 were beyond the experiment time and were not determined. This study revealed that the half-lives of chitosan materials varied from several days to months as a function of DD, which will be important when preparing chitosan-based biomedical materials with pre-determined degradation rate.

Little variation in water absorption was observed for Chi93, Chi81, and Chi71 samples after 3 days immersion with the equilibrium water content of 100%, 130%, and 220%, respectively (Fig. 6), while for matrices Chi62, Chi56, and Chi52, the kinetics of water absorption was very different. The values increased during the first 7 days and were constant for the next several days with values of 300%, 450%, and 650%, respectively, and then another increase occurred (Fig. 6). This was especially true for Chi52 and Chi56, when the increase began after 11 and 21 days, respectively, which were consistent with the time when the samples lost half their weight (Fig. 4). Thus, the equilibrium water absorption curve of chitosan matrices had a profile similar to the weight loss profile. Figure 7 shows a comparison of the results from Figures 4 and 6, which shows a 'tri-phasic' pattern that differs for the three matrices. For Chi62, there was a continuous weight loss until day 7 (10%) (Phase 1), followed by a slower weight loss until day 21 (Phase 2), then followed by another acceleration until the matrix completely broke down (Phase 3). The water absorption

Table 3. Degradation half-lives of chitosan matrices in vitro. N/D refers to 'not determined' as the half-life is beyond the experiment time (84 days)

Matrices code	Weight half-life (days)	M_w Half-life (days)
Chi93	N/D	N/D
Chi81	N/D	N/D
Chi71	N/D	N/D
Chi62	>56	20.0
Chi56	27.3	8.8
Chi52	9.8	8.4

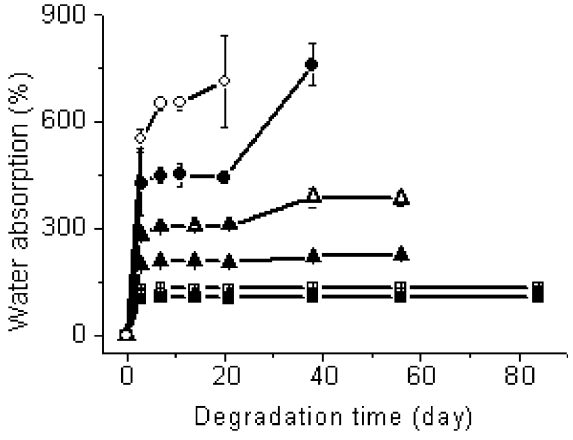

Figure 6. Change in water absorption of chitosan matrices undergoing in vitro degradation by lysozyme: (■) Chi93; (□) Chi81; (▲) Chi71; (△) Chi61; (●) Chi56; (○) Chi52.

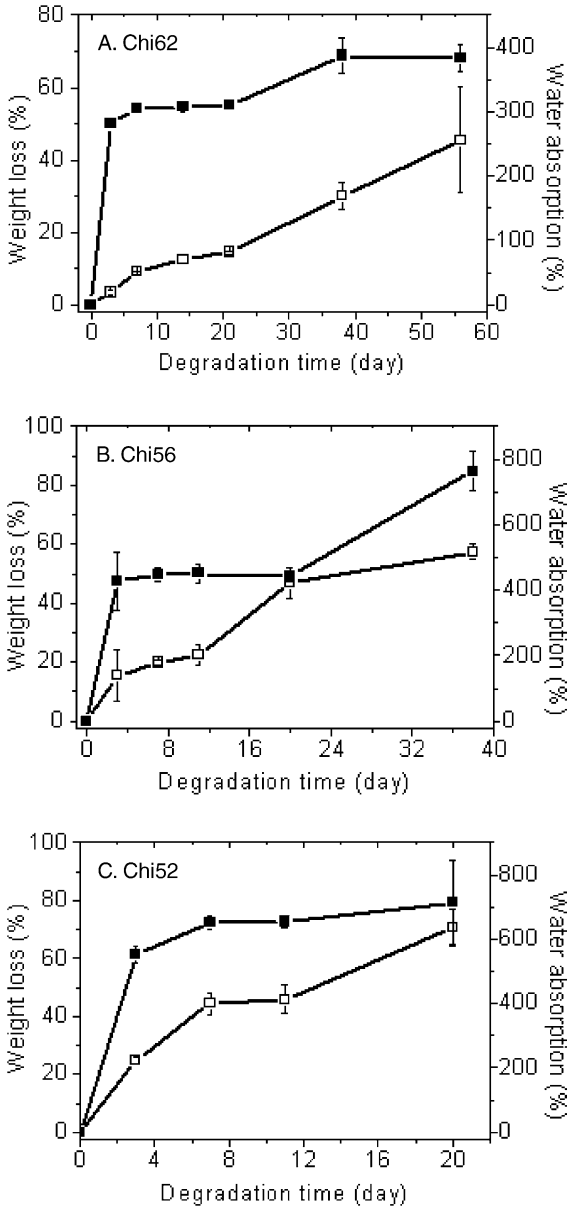

Figure 7. Percent weight loss (□) and water absorption (■) of chitosan matrices over degradation time.

also appeared to have three stages, similar to the weight loss (Fig. 7A). A similar pattern also existed for Chi56 and Chi52, but with an earlier Phase 3, beginning at day 11 (Fig. 7B and C). This is the first report of this 'tri-phasic' degradation of chitosan. This might result from the crystallinity of chitosans (Fig. 3) because of the coexistence of the crystalline and amorphous zones in chitosan macromolecules. *N*-Acetylation of HDC under homogeneous conditions caused more amorphous regions in chitosan molecules, which would be more water permeable and in turn more accessible to lysozyme than the crystalline zones. The former undergoes more rapid water adsorption and then degradation than does the latter. Therefore, the first phase of the degradation process exhibits rapid weight loss and water adsorption, which is then followed by a relatively static stage, in which the lysozyme and water permeate into the crystalline zone, which is more difficult. The crystalline zone is then degraded by lysozyme gradually, and thus the third phase develops more slowly.

The swelling of chitosan matrices reached a maximum by day 3 with values of 208%, 235%, 265%, 393%, 460%, and 530% for Chi93, Chi81, Chi71, Chi62, Chi56, and Chi52, respectively (Fig. 8). The swelling degree increased as the DD of the chitosan matrices decreased, which is comparable to those reported by Aiba.[4] However, in Aiba's work, the results were obtained by comparing the length of chitosan films in ethanol and in water, while in our study the swelling property was investigated under more physiological conditions. Furthermore, it was found that for Chi93, Chi81, and Chi71, the swelling degrees were almost constant throughout the degradation. In contrast, for Chi62, Chi56, and Chi52 shrinkage was observed after day 3 of the degradation and the swelling degrees decreased to 310% and 297% for Chi56 and Chi52, respectively.

In addition to the weight changes shown in Figure 4, it was found that the weight loss of chitosan matrices be-

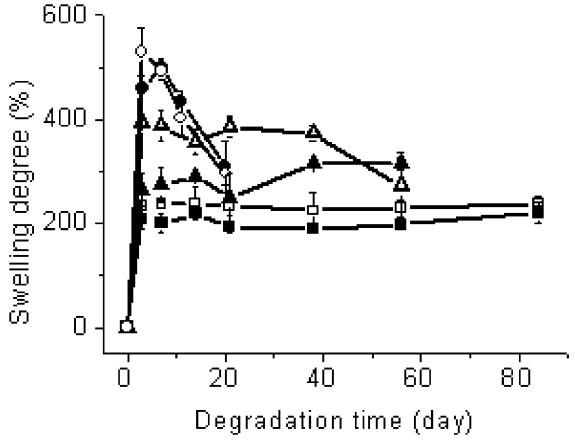

Figure 8. Change in swelling degree of chitosan matrices undergoing in vitro degradation by lysozyme: (■) Chi93; (□) Chi81; (▲) Chi71; (△) Chi61; (●) Chi56; (○) Chi52.

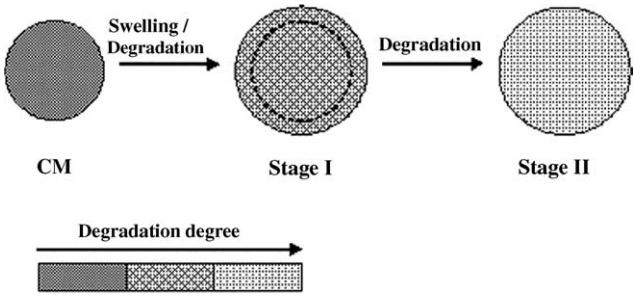

Figure 9. Schematic illustration of the swelling and degradation process of chitosan matrices (CM).

longed to bulk erosion as described by Gopferich and co-workers.[33] Because, chitosan is hydrophilic and diffusion of water into chitosan matrices is faster than degradation, the matrices begin to swell prior to degradation. The degradation process of chitosan matrices is divided into two stages in our study as shown in Figure 9. When chitosan matrices were immersed into the lysozyme solution, water permeated into the matrices making them swell. Accompanying the hydration process, bond cleavage and degradation of chitosan occurred. Stage I was such a process in which swelling and degradation both existed, but the former surpassed the latter, resulting in the continued swelling of matrices, as was seen during the first three days of degradation for Chi52 and Chi56. When swelling reached a maximum, continual degradation led to weight loss (Stage II), the matrices tended to be thinner, and the swelling degree decreased, as happened after 7 days for Chi52 and Chi6. It was concluded that the dimension of chitosan matrices depended on the relationship of swelling and degradation. The results could help when designing chitosan-based drug delivery systems, tissue scaffolds, and engineered bone and cartilage.

Figure 5 presents the profiles of initial degradation rate, equilibrium water absorption, and swelling degree as a function of the DD of chitosans. This figure clearly shows the dependence of the initial degradation rate, equilibrium water absorption, and swelling degree of chitosan matrices on the DD. All the values increased with lower DD and acceleration dramatically when the DD decreased below 71.7%. This indicates that *N*-acetylation of HDC under homogeneous conditions made chitosans less crystalline and more susceptible to lysozyme as described previously by Aiba.[4,20]

3.3. Molecular weight and polydispersity index

The weight average molecular weight (M_w) and the polydispersity (PI) were determined by gel permeation chromatography (GPC). The M_w of all chitosan matrices decreased exponentially with degradation time except Chi93 (Fig. 10). By 21 days, the M_w of Chi81, Chi71, Chi62, Chi56, and, Chi52 decreased to 90%,

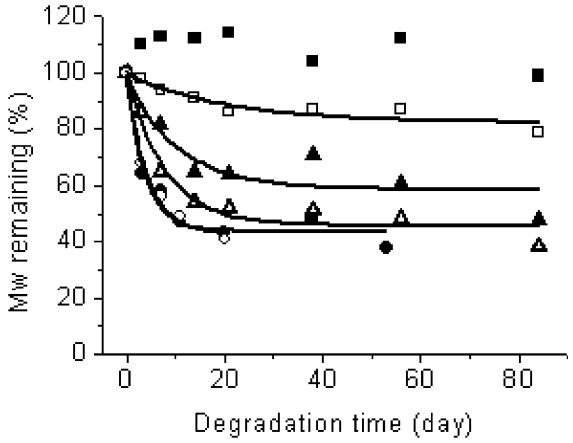

Figure 10. Change in M_w of chitosan matrices undergoing in vitro degradation by lysozyme: (■) Chi93; (□) Chi81; (▲) Chi71; (△) Chi61; (●) Chi56; (○) Chi52.

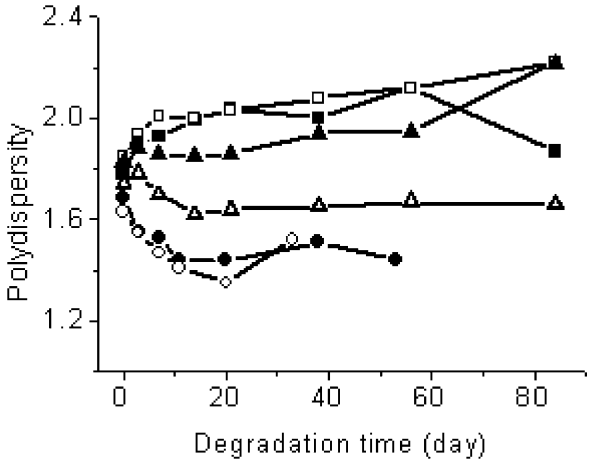

Figure 11. Change in polydispersity of chitosan matrices undergoing in vitro degradation by lysozyme: (■) Chi93; (□) Chi81; (▲) Chi71; (△) Chi61; (●) Chi56; (○) Chi52.

65%, 50%, 45%, and 40% of the original M_w, respectively. This was consistent with the tendency of their weight loss with some differences (Fig. 4). The M_w half-lives of the chitosan matrices were 8.4, 8.8, and 20 days for Chi52, Chi56, and Chi62, respectively, and the M_w half-lives of Chi71, Chi81, and Chi93 were not determined as they were longer than our experimental study (Table 3). Significant differences in half-life were found between weight and molecular weight during the degradation process for Chi52, Chi56, and Chi62, for which the M_w half-life was shorter than the weight half-life. The time distance increased as the DD of the matrices increased, 1.4 days for Chi52, 18.5 days for Chi56, and more than 36 days for Chi62. In the degradation process, when lysozyme acts on chitosan the cleavage of the β-(1→4) glycosidic bonds occurs, thus reducing the molecular weight. Most of the broken chains are retained in the bulk material until the bond cleavage continues to the point where fragments of low M_w are produced that dissolve into the surrounding media, in turn resulting in weight loss. Our results confirmed Freier's conclusion about the influence of the DD on the degradation of chitosan.[15] Therefore, the relationship of M_w loss and weight loss of chitosan matrices should be taken into consideration when selecting or preparing chitosans as candidates for engineering bone and cartilage.[31]

However for Chi93, the M_w experienced a surprising increase during degradation (Fig. 10), compared with the unchanged weight (Fig. 4). According to previous reports, chitosan with DD above 90% could not be degraded by lysozyme,[6,7,20–22] but no data on the change of molecular weight were reported. From other types of chitosan samples with high DD above 90% in our laboratory, this tendency was also seen (data not shown). It has been proposed that this perplexing increase probably occurs due to glycoprotein bonding of chitosan with lysozyme.[32]

The PI of all chitosan matrices exhibited changes during degradation (Fig. 11). For Chi62, Chi56, and Chi52, the decrease in PI values was due to the obvious decrease in M_w and the release of low molecular weight degradation products into surrounding media (Fig. 11). For Chi81 and Chi71, the PI appeared to increase because the degradation rate was relatively low and most of the degradation products in higher M_w remained in the matrices.

3.4. Reducing sugar

The amount of reducing sugar in the supernatant was determined with ferricyanide. As shown in Figure 11, the concentrations of reducing sugar in supernatant were only 85, 89, and 103 μg/mL for Chi93, Chi81, and Chi71, respectively, at 7 days and almost remained unchanged through the degradation. Concentrations of 140, 216, and 365 μg/mL were obtained for Chi62, Chi56, and Chi52, respectively, at 7 days and increased dramatically after 20 days (Fig. 12). Compared with Figure 9, in which molecular weight of Chi62, Chi56, and Chi52 decreased during the first 20 days, it was found that decreasing molecular weight and increasing concentration of reducing sugar appeared in different degradation phases. When chitosans are degraded by lysozyme, the cleavage of β-(1→4)-glycosidic bonds occurs, and the degradation products, including chitosans with lower molecular weight, chitogligomers, and *N*-acetyl-D-glucosamine residues, may be released and dissolved into the degradation media. Therefore, the molecular weight decreases during the first 20 days. The degradation products may be further degraded by lysozyme in the degradation media and more reducing sugar is released, which causes an obvious increase of the concentration of reducing sugar after 20 days. These results indicate that the change of molecular weight can reflect

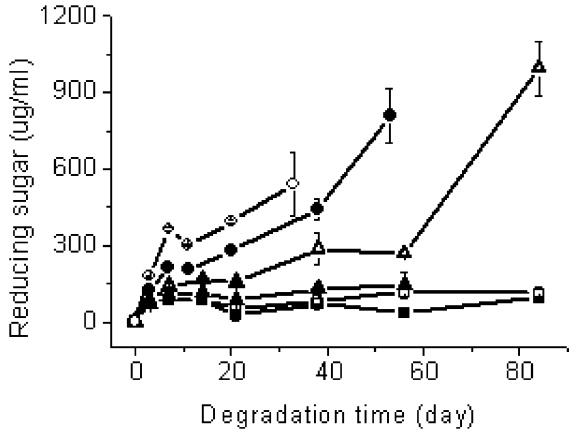

Figure 12. Change in reducing sugar content in the supernatant undergoing in vitro degradation by lysozyme: (■) Chi93; (□) Chi81; (▲) Chi71; (△) Chi61; (●) Chi56; (○) Chi52.

the degradation status of chitosans more precisely than the concentration of reducing sugar in the supernatant.

4. Conclusion

Chitosan matrices with various degrees of *N*-acetylation were investigated during lysozymic degradation in vitro by measuring weight loss, water absorption, swelling, molecular weight of bulk material, and also reducing sugar content in media. It was found that chitosan matrices with DD of 93.5%, 81.7%, and 71.7% experienced very slow degradation process, while those with DD of 62.4%, 56.1%, and 52.6% underwent significant degradation. The matrices half-lives based on weight and M_w decreased significantly with decreasing DD of chitosan. In addition, a 'tri-phasic' pattern of weight loss and water absorption was observed during the degradation process of chitosan matrices with DD of 62.4%, 56.1%, and 52.6%. Finally, chitosan matrices with various DD exhibited different swelling behaviors during the degradation process. These results made it possible to control not only the degradation rate but also the swelling degree of chitosans during the degradation process.

Acknowledgments

The authors would like to acknowledge funding support from the National High-Tech Program of China (No. 2001AA326020) and the National Basic Research Program of China (No. 2002CB713804).

References

1. Illum, L. *Pharm. Res.* **1998**, *15*, 1326–1331.
2. Chang, K. L. B.; Tsai, G.; Lee, J.; Fu, W. R. *Carbohydr. Res.* **1997**, *303*, 327–332.
3. Hirano, S.; Tsuchida, H.; Nagao, N. *Biomaterials* **1989**, *10*, 574–576.
4. Aiba, S. *Int. J. Biol. Macromol.* **1991**, *13*, 40–44.
5. Gaserod, O.; Smidsrod, O.; Skjak-Brak, G. *Biomaterials* **1998**, *19*, 1815–1825.
6. Lee, K. Y.; Ha, W. S.; Park, W. H. *Biomaterials* **1995**, *16*, 1211–1216.
7. Tomihata, K.; Ikada, Y. *Biomaterials* **1997**, *18*, 567–575.
8. Onishi, H.; Machida, Y. *Biomaterials* **1999**, *20*, 175–182.
9. Agnihotri, S. A.; Mallikarjuna, N. N.; Aminabhavi, T. M. *J. Controlled Release* **2004**, *100*, 5–28.
10. Yu, W. T.; Xiong, Y.; Liu, X. D.; Wang, W.; Xie, W. Y.; Ma, X. J. *Chem. Res. Chin. Univ.* **2004**, *25*, 1381–1383.
11. Chenite, A.; Chaput, C.; Wang, D.; Combes, C.; Buschmann, M. D.; Hoemann, C. D.; Leroux, J. C.; Atkinson, B. L.; Binette, F.; Selmani, A. *Biomaterials* **2000**, *21*, 2155–2161.
12. Yamaguchi, I.; Itoh, S.; Suzuki, M.; Sakane, M.; Osaka, A.; Tanaka, J. *Biomaterials* **2003**, *24*, 2031–2036.
13. Kim, S. E.; Park, J. H.; Cho, Y. W.; Chung, H.; Jeong, S. Y.; Lee, E. B.; Kwon, I. C. *J. Controlled Release* **2003**, *91*, 365–374.
14. Lee, J. E.; Kim, K. E.; Kwon, I. C.; Ahn, H. J.; Lee, S. H.; Cho, H.; Kim, H. J.; Seong, S. C.; Lee, M. C. *Biomaterials* **2004**, *25*, 4163–4173.
15. Xu, H. H. K.; Simon, C. G., Jr. *Biomaterials* **2005**, *26*, 1337–1348.
16. Babense, J. E.; Anderson, J. M.; McIntire, L. V.; Mikos, A. G. *Adv. Drug. Delivery Rev.* **1998**, *33*, 111–139.
17. Varum, K. M.; Myhr, M. M.; Hjerde, R. J. N.; Smidsrod, O. *Carbohydr. Res.* **1997**, *299*, 99–101.
18. Henry, J. B. *Clinical Diagnosis and Management by Laboratory Methods*, 18th ed.; Saunerders: Philadelphia, 1991; pp 1371.
19. Temel, A.; Kazokoglu, H.; Taga, Y. *Ann. Ophthalmol.* **1991**, *23*, 191–194.
20. Aiba, S. *Int. J. Biol. Macromol.* **1992**, *14*, 225–228.
21. Nordtveit, R. J.; Varum, K. M.; Smidsrod, O. *Carbohydr. Polym.* **1994**, *23*, 253–260.
22. Nordtveit, R. J.; Varum, K. M.; Smidsrod, O. *Carbohydr. Polym.* **1996**, *29*, 163–167.
23. Freier, T.; Koh, H. S.; Kazazian, K.; Shoichet, M. S. *Biomaterials* **2005**, *26*, 5872–5878.
24. Chang, K. L. B.; Lin, J. *Carbohydr. Polym.* **2000**, *43*, 163–169.
25. Broom, N. D.; Oloyede, A. *Biomaterials* **1998**, *19*, 1179–1188.
26. Yamaguchi, I.; Itoh, S.; Suzuki, M.; Osaka, A.; Tanaka, J. *Biomaterials* **2003**, *24*, 3285–3292.
27. Khor, E. *Chitin: Fulfilling a Biomaterials Promise*; Elsevier: Amsterdam, 2001; pp 127–130.
28. Prashanth, K. V. H.; Kittur, F. S.; Tharanathan, R. N. *Carbohydr. Polym.* **2002**, *50*, 27–33.
29. Imoto, T.; Yagishita, K. *Agric. Biol. Chem.* **1971**, *35*, 1154–1156.
30. Samuels, R. J. *J. Polym. Sci., Part B: Polym. Phys.* **1981**, *19*, 1081–1105.
31. Hutmacher, D. W. *Biomaterials* **2000**, *21*, 2529–2543.
32. Roberts, G. A. F. *Chitin Chemistry*; Macmillan: London, 1992; pp 238–241.
33. von Burkersroda, F.; Schedl, L.; Gopferich, A. *Biomaterials* **2002**, *23*, 4221–4231.