Molecular Stability of Chitosan in Acid Solutions Stored at Various Conditions

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Received 27 November 2006; accepted 20 August 2007
DOI 10.1002/app.27376
Published online 13 November 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The stability of chitosan with a degree of deacetylation (DD) of 88 and 81% was investigated in solution during storage for 60 days at various temperatures (60, 28, and 5°C) and acid concentrations (0.8*M*, 0.2*M*, and 0.1*M*). The first-order rate constant of chain hydrolysis of 88%DD chitosan at 60°C was about 1.4 times higher than that of the 81%DD sample. At 28°C, the rates of hydrolysis for both chitosan samples were four to five times lower than those at 60°C and are similar. At 5°C, chain degradation was not significant. Although acetic acid caused significantly higher ($P \leq 0.05$) chain scission than formic acid, no significant difference of rate change was observed among three different acid concentrations. Reprecipitation of dissolved chitosan was applied for its purification and to transfer dissolved chitosan to the solvent used to measure its molecular weight. Reprecipitation resulted in slightly lower molecular weight ($P \leq 0.05$) for both 88%DD and 81%DD samples. The molecular weight of chitosan before and after reprecipitation had good linear relationship ($r^2 > 0.9$). © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 2588–2593, 2008

Key words: acetic acid; chitosan; formic acid; rate of hydrolysis

INTRODUCTION

Chitin is a naturally abundant skeletal aminopolysaccharide of many arthropods. It is a copolymer of β-(1-4)-linked *N*-acetyl-D-glucosamine and D-glucosamine and is difficult to dissolve. Its deacetylated derivative chitosan can readily be dissolved in dilute organic acids, forming a typical non-Newtonian, shear-thinning fluid.[1,2]

Chitosan has remarkable characteristics of cationicity, biodegradability and biocompatibility; it can be produced as beads, gel, membrane, or fibers and has many potential applications.[3–5] Products in the market include Tegasorb™ (3M, USA) and Chitopack (Eisai, Japan) for wound-healing, Chitopearl (Fujibo, Japan) for anion exchange chromatography, Syvek NT Patch (Marine Polymer Technologies, USA) and Chito-Seal (Abott Vascular Devices, USA) for hemostatic dressing, and chitosan hard capsules (Aicello Chemical, Japan) for lower GI tract drug delivery.

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Contract grant sponsor: Swedish International Development Cooperation Agency (Sida).

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This article deals with the hydrolysis of chitosan, as it occurs after dissolution and storage at various conditions in dilute organic acids. The hydrolysis of chitin and chitosan so far has been studied mainly with the aim of producing low molecular weight chitosan products. Enzymatic hydrolysis of the macromolecular chitosan in solution can be achieved, and it results in various oligochitosans, depending on the enzyme used.[6–8] Chemical degradation can be accomplished by various acids depending on the type of chitosan and its concentration, the degree of deacetylation, type of acid, treatment time, and temperature. Data are available for acid hydrolysis by hydrochloric, nitric, phosphoric, hydrofluoric, and sulfuric acids.[9–14] Liquid crystalline chitin can also be prepared by hydrolyzing chitin with hydrochloric acid.[15]

Although dissolution of chitosan in diluted acetic acid is the start of most applications of chitosan, only limited data are available on the degradation kinetics of chitosan in organic acids. Chen et al. have reported that the hydrolysis of chitosan in acetic acid solution is high in samples with relatively high acetyl content and molecular weight.[16,17] The same phenomenon has been observed for chitosan in lactic acid solutions.[18] Ascorbic acid was found to induce more

rapid degradation at low polymer concentration.[19] This article presents the long term degradation kinetics of chitosan in acetic and formic acid solution as analyzed by viscometry and gel permeation chromatography.

MATERIALS AND METHODS

Chitosan samples with a 88 and 81% degree of deacetylation (DD) were prepared from shrimp chitin at Bioprocess Technology, Asian Institute of Technology, Thailand.[20] The %DD of the samples was determined by the acid hydrolysis-HPLC method.[21]

The residual protein and ash content of the samples were below 1 and 0.6%, respectively. All chemicals used were of analytical grade, purchased from Carlo Erba unless otherwise specified.

Dissolution and storage of chitosan solutions

Aliquots of vacuum-dried chitosan (1 g) were dissolved in 100 mL of 0.2*M* acetic acid [close to 1% (v/v) acetic acid] using an orbital shaker (100 rpm) at room temperature for 24 h. Then the solutions were stored at various temperatures: 60, 28, and 5°C for various periods of time: 0, 10, 20, 40, and 60 days in air-tight polypropylene bottles.

Later, to observe the effect of the acid type and their concentrations, the storage temperature was kept constant at 28°C. Chitosan (1 g) was dissolved in 0.1*M*, 0.2*M*, and 0.8*M* acetic acid and formic acid at same conditions as mentioned above. The solutions were then stored for 0, 10, 20, 40, and 60 days.

Determination of intrinsic viscosity and viscosity-average molecular weight (M_v) of chitosan in solution

The intrinsic viscosity of the chitosan solutions was measured in 0.2*M* acetic acid/0.1*M* sodium acetate buffer using a Cannon-Fenske capillary viscometer immersed in a Uni-thermal Bath (Yamato, model BR-61) at 30 ± 0.01°C. All chitosan solutions were filtered through a 0.45-μm membrane filter before analysis. The intrinsic viscosity was determined from reduced and inherent viscosities at infinite dilution (Fig. 1). For further assessment of the viscosity-average molecular weight, the intrinsic viscosity was determined from the reduced viscosity using the Mark–Houwink equation:

$$[\eta] = kM_v^a$$

where M_v is the viscosity-average molecular weight, $[\eta]$ is the intrinsic viscosity, and k and a are the constants of values 1.424×10^{-5} (dL/g) and 0.96, respectively.[22]

Because chitosan was dissolved and stored in various acid solutions and intrinsic viscosity was determined in the acetate buffer, the storage solution was adjusted to measurement medium: 0.2*M* acetic acid and 0.1*M* sodium acetate, prior to the measurement.

For chitosan stored in formic acid solution, chitosan was first reprecipitated by dropwise addition of 1*M* NaOH under vigorous stirring until pH of the solution reached 11.5. The precipitate was washed and soaked several times with deionized water to attain neutral pH. Then, the precipitate was air-dried followed by vacuum-drying. The dried chitosan was dissolved in the measurement medium to determine the intrinsic viscosity.

To check the effect of this extra precipitation, chitosan in acetic acid solutions was also reprecipitated by the same procedure as used for chitosan stored in formic acid solutions. Then the viscosity-average molecular weight was obtained. The properties of chitosan with and without reprecipitation were compared.

Determination of weight-average molecular weight (M_w) and polydispersity index by gel permeation chromatography

Chitosan (0.1% w/v) was dissolved overnight in 0.2*M* acetic acid/0.1*M* sodium acetate. The solution was filtered through a 0.45-μm membrane filter and auto-injected (150 μL) into a Waters™ HPLC system. The system was equipped with Waters™600 Controller, Waters™ Ultra-Hydrogel 500, 1000, 2000 GPC columns, and Waters™410 refractive index detector. The samples were eluted with the same solvent at a flow rate of 0.6 mL/min. The temperature of the column oven was 55°C and the sample compartment was 25°C. The calibration curve for the determination of molecular weight was prepared by using dextran standards of 9.9, 17.9, 35.6, 74.3, 170, 230, 535, and 200 kDa (Phenomenex, USA). The polydispersity index (PDI) was calculated by dividing the weight-average molecular weight (M_w) by the number-average molecular weight (M_n).

The data presented here are the average of duplicate observations. Analysis of variance was performed to determine the significance of the differences using SPSS software.

RESULTS AND DISCUSSION

Effect of storage temperature and duration on the stability of chitosan solutions

In research and in many chitosan products, chitosan is used in the dissolved state. In this study, the stability of chitosan with a degree of deacetylation of 88 and 81% was investigated in various solutions

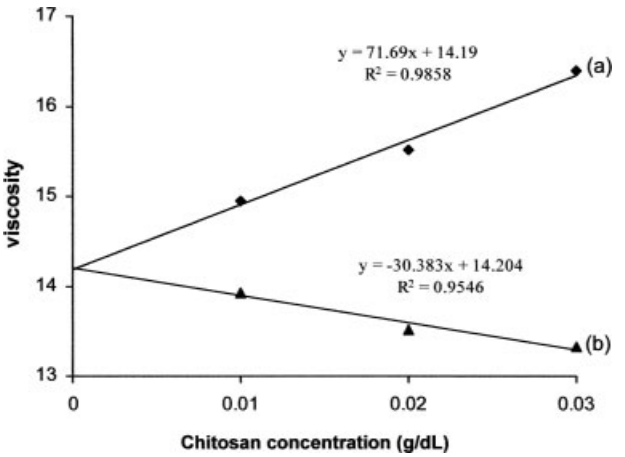

Figure 1 Example of the calculation of intrinsic viscosity from (a) reduced viscosity and (b) inherent viscosity of a 81%DD chitosan solution stored at 5°C for 60 days [chitosan solution: 1% (v/w) chitosan dissolved in 0.2*M* acetic acid].

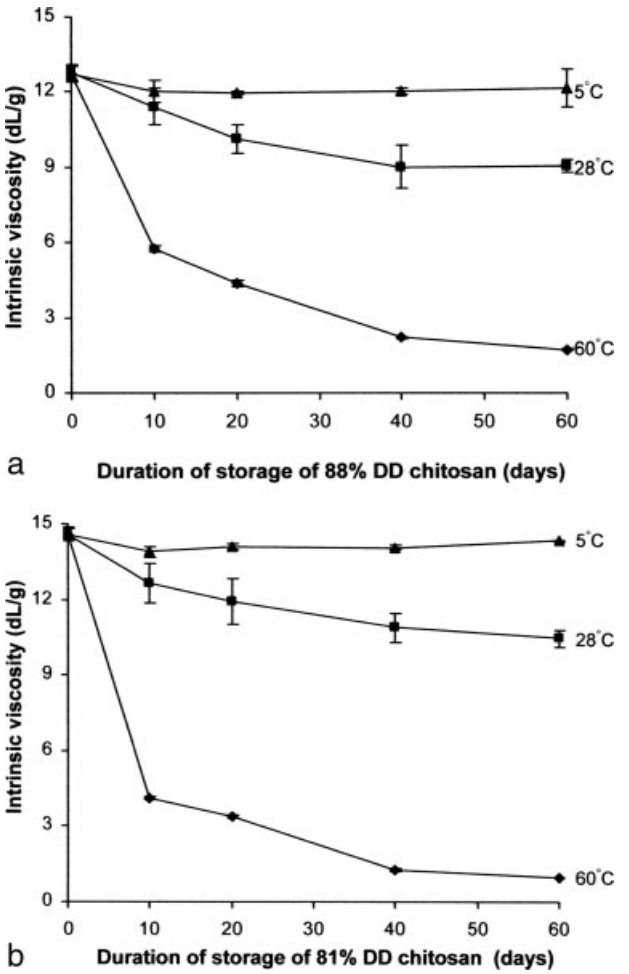

Figure 2 Effect of storage temperature and duration on the intrinsic viscosity of (a) 88%DD and (b) 81%DD chitosan samples [chitosan solution: 1% (w/v) chitosan solution dissolved in 0.2*M* acetic acid].

and at various temperatures. Chitosan 1% (w/v) was prepared in 0.2*M* acetic acid and stored at 5, 28, and 60°C. It was observed for both chitosan preparations (Fig. 2) that the higher temperature and the longer storage time resulted in more hydrolysis on the chitosan chain. The most pronounced chain degradation of chitosan was observed at 60°C for both chitosan preparations. A sharp drop (more than 50% of the initial value) in intrinsic viscosity occurred within first 10 days. However, no significant chain degradation was observed in the solutions kept at 5°C till 60 days ($P \leq 0.05$). The rate of hydrolysis followed first-order rate kinetics (Fig. 3). This finding is in agreement with the hydrolysis data for chitosan hydrolyzed with hydrochloric acid.[14]

The PDI of the samples has been studied in detail. The PDI of 81%DD chitosan increased from 2.39 to 4.11 after 20 days of storage. Later, it gradually decreased to 1.76 with increasing storage time. This trend was found for both chitosan samples, but the change was more pronounced in the 81%DD preparation (Fig. 4). The increase in PDI is probably due to a preferential scission in longer chitosan chains leading to more heterogeneity in the molecular weight distribution. After a prolonged period of time, more and more scissions occurred to give small molecular chains, which results in more homogeneity of the length of chain and lower PDI. The decrease of PDI with longer treatment time was also found when chitosan solution was subjected to sonication,[17] but the increase of PDI was not observed in that case. The reason could be that the chain degradation caused by sonication occurred too fast, and an intermediary increase of PDI could not be observed. The change in polydispersity was not observed at lower temperatures (data not shown).

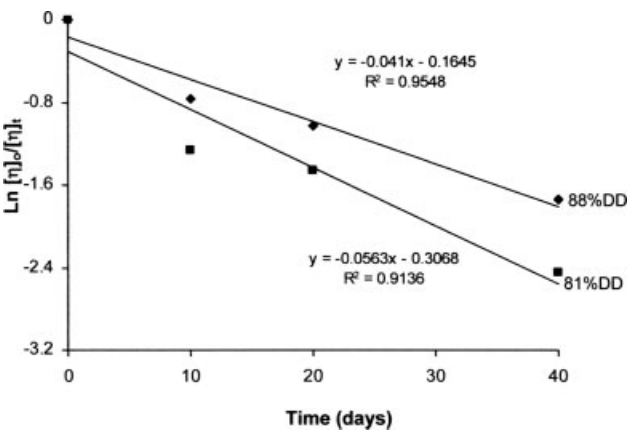

Figure 3 Degradation kinetics of chitosan in 0.2*M* acetic acid at 60°C.

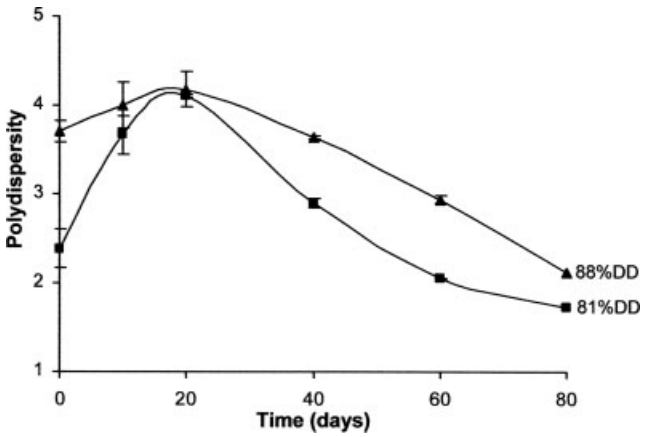

Figure 4 Change of polydispersity upon degradation of chitosan in 0.2*M* acetic acid at 60°C.

The first-order degradation rate constants of two chitosan samples at 60°C are four to five times higher than those at 28°C (Fig. 5). The rate of degradation of 88%DD was significantly faster than that of 81%DD in 0.2*M* acetic acid at 60°C ($P \leq 0.05$). It is consistent with the observation that the first-order rate of degradation of lower %DD chitosan in hydrochloric acid is higher than that of higher %DD chitosan.[14] It might be that, in 81%DD chitosan, the longer acetylated segments are more prone to hydrolysis[17] compared to shorter acetylated segments of 88%DD. In lactic acid solution, chitosan with a higher molecular weight and lower %DD showed to be hydrolyzed faster than its opposite counterparts.[18] However, this effect was not significant ($P \leq 0.05$) when chitosan in 0.2*M* acetic solution was stored at 28 or 5°C (Fig. 5).

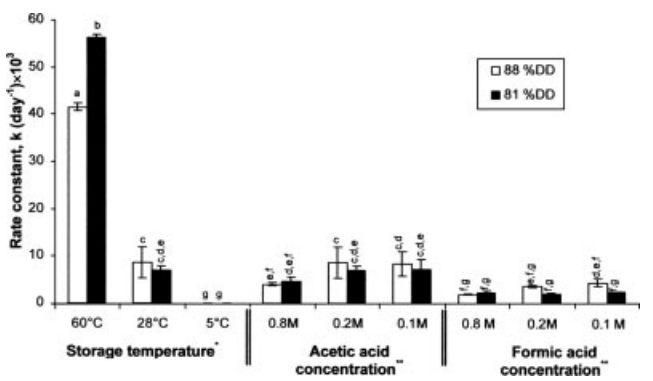

Figure 5 Effect of storage temperature, acid type, and concentrations on the rate of chitosan degradation ($-d[\eta]/dt = k[\eta]_o$). Storage conditions: *1% (w/v) chitosan solution in 0.2*M* acetic acid for different temperature experiments; **1% (w/v) chitosan solution at 28°C for different acid type and concentrations experiments. ^{a,b,c,d,e,f,g}Different letters indicate that the rate constants are different at $P \leq 0.05$.

Effect of acid type and concentration on the stability of chitosan solution

Chain degradation depends on the type of acid used and on the acid concentration. Degradation has been compared for acetic and formic acid at three different concentrations (Fig. 5). Chitosan solutions were more stable when chitosan is dissolved in formic acid ($P \leq 0.05$) than in acetic acid. However, the rates of degradation were not significantly different ($P \leq 0.05$) when acid concentrations were changed from 0.1*M* to 0.8*M* for the same acid type at 28°C. Moreover, no difference in degradation rate was observed between 88%DD and 81%DD samples. This indicates that degradation rate of chitosan in acetic and formic acid solutions is not affected by the change of %DD (7 units) and molecular weight (300 kDa) when storage temperature is lower than 28°C and the acid concentration is lower than 0.8*M*. Consequently, no significant change in PDIs was found under these experimental conditions.

Effect of reprecipitation of chitosan from acetic acid solutions

Solid chitosan should be purified by dissolution and removal of insoluble particles, followed by reprecipitation. This reprecipitation is carried out by neutralizing chitosan solution with alkali. It is known that this reprecipitation process does not change the degree of deacetylation of polymeric chitosan.[23] This study showed that reprecipitated chitosan samples had slightly lower ($P \leq 0.05$) molecular weight than the original samples in the range of 1000–1800 kDa. Moreover, a good correlation ($r^2 > 0.9$) was observed between the original and reprecipitated chitosan (Fig. 6).

Relationship between weight-average molecular weight (M_w) and viscosity-average molecular weight (M_v)

Molecular weight is one of the most important physical properties of chitosan. Generally, hydrodynamic volume of the molecular chain is measured in the presence of an electrolyte such as sodium acetate, sodium chloride, and lithium chloride. Calibration with the molecular weight resulted from laser light scattering measurement; viscosity-average molecular weight can be calculated from intrinsic viscosity using the Mark–Houwink relation. The Mark–Houwink constants vary with the type of electrolyte, electrolyte concentration, and the degree of deacetylation of chitosan. As a result, different solvent systems give different molecular weight values for same chitosan sample. No relationship between different solvent systems was found.[24] Because of the instrument availability and relatively easier operation, gel permeation chromatography is popular

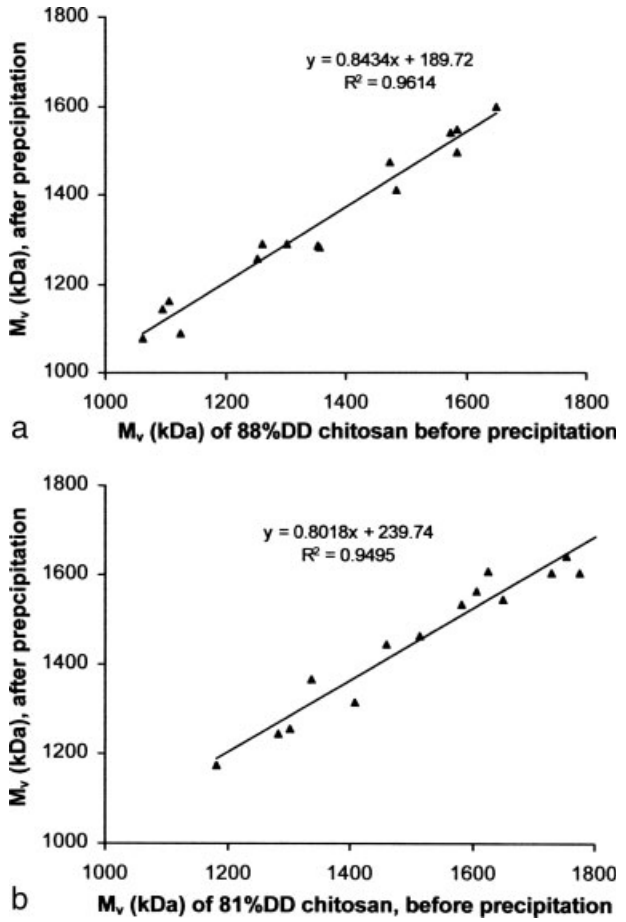

Figure 6 Relationship between the viscosity-average molecular weights of (a) 88%DD and (b) 81%DD chitosans before and after reprecipitation from different concentrations of acetic acid stored at 28°C.

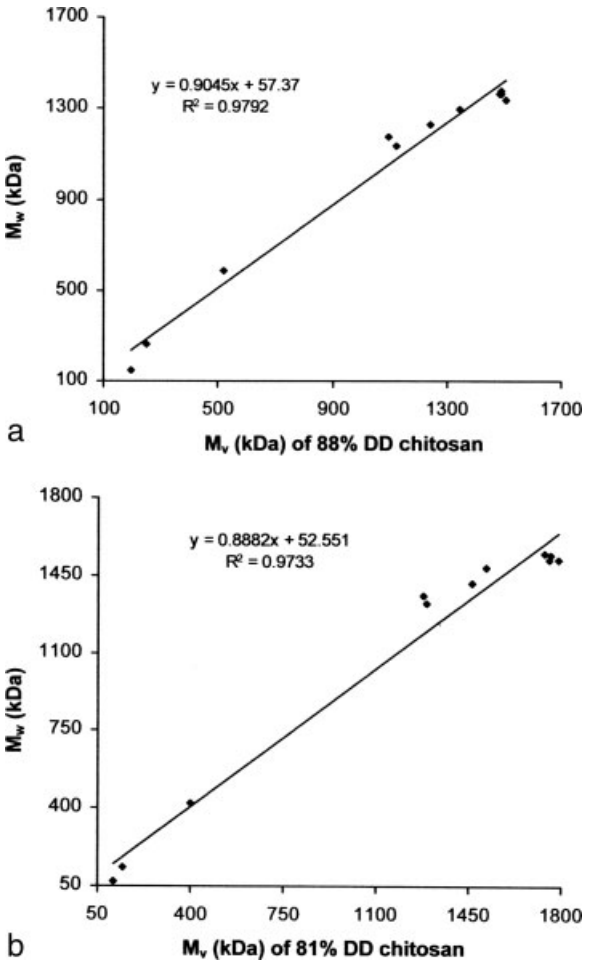

Figure 7 Relationship between M_v from capillary viscometer measurement and M_w from gel permeation chromatography using dextran as standard (a) 88%DD and (b) 81 %DD chitosan samples.

among chitin scientists. However, there are no chitosan molecular weight standards available in market. So, calibration is commonly done using dextran or pullulan standards. Consequently, the resultant molecular weight measured by the GPC method gives values relative to the standard used. Furthermore, the different vision among the researchers regarding the choice of the solvent systems makes the research data exchange even more difficult. The use of pullulan standards to access the molecular weight of chitosan is considered to be a good approximation, but needs further validation.[25]

Recently, MALDI-TOF has been used to sequence chitooligomers, and therefore exact molecular weight independent of solvent systems can now be measured. But, for higher molecular weight determination, the lack of a suitable matrix still posts a problem for a meaningful exchange of data. In this study, the solvent system 0.2*M* acetic acid/0.1*M* sodium acetate buffer was used both for intrinsic viscosity and GPC measurement. Dextran standards were used in GPC measurement. Figure 7 shows a good linear relation ($r^2 > 0.9$) between M_v and M_w of chitosan, although M_v is slightly higher than M_w in the testing range of 50–1800 kDa.

CONCLUSION

Capillary viscometer and gel permeation chromatography were used to assess the stability of 1% (w/v) chitosan (88%DD and 81%DD) solutions in acetic acid and formic acid. The rate of degradation of chitosan in acid solutions followed first-order rate kinetics. The rate was faster when storage temperature increased, but different acid concentrations (0.8*M*, 0.2*M*, and 0.1*M*) did not affect the rate. Chitosan in acetic acid solution stored in 5°C did not cause chain degradation over 60 days. Chitosan is more stable in formic acid than in acetic acid solution. Purification of chitosan by 1*M* NaOH did have a small effect if any on the molecular stability of

chitosan in acetic acid solution. The relationship between M_v and M_w was found to be good under the tested solvent system.

The authors thank Prof. Suwalee Chandrkrachang, Dr. Josef Tränkler, and Dr. Suwabun Chirachanchai for helpful comments.

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