Effect of Recovery Methods and Conditions on the Yield, Solubility, Molecular Weight, and Creep Compliance of Regenerated Chitosan

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Received 16 January 2001; accepted 7 July 2001

ABSTRACT: The objective of this study is to explore the effect of using different recovery methods and conditions on the yield, solubility, molecular weight, and creep compliance of the regenerated chitosan. The results show that yields obtained by dialysis were higher than those using recovery medium of alkali solutions, organic solvents, or alkali–alcohol–water mixtures. For those chitosans employing alkali solutions as the recovery medium, the higher the alkali concentration used, the higher the yields obtained, although the total quantity of alkali in the solution were the same. Solubilities of regenerated chitosans were similar and independent at the methods of using alkali solution, organic solvent or alkali–alcohol–water mixture or at different concentrations of alkali solution. The molecular weight of regenerated chitosan decreased from 2.37×10^7 to 1.68×10^7 Da proportionally with the concentration of the alkali solution of the recovery medium from 1N to 8N. Creep compliance of regenerated chitosan gel obtained from 65% degree of deacetylation (DD) chitosan was lower than that of either 72 or 89% DD chitosan gel. Of the same DD chitosan, compliance of regenerated chitosan gels obtained by using a higher concentration of alkali solution was lower than that of a lower concentration ones. Hydrogels regenerated from different DD chitosans and/or different recovery mediums have different structure and tactile properties. Therefore, they can be used as wound dressings suited to different applications. © 2002 John Wiley & Sons, Inc. J Appl Polym Sci 84: 193–202, 2002; DOI 10.1002/app.10296

Key words: chitosan; regenerated; creep compliance; molecular weight; solubility

INTRODUCTION

Chitin, the second-most abundant biopolymer, and its deacetylated product, chitosan, are high-molecular-weight biopolymers and are recognized as versatile, environmentally friendly raw materials.[1] There are many applications for these chitinous materials including use in agriculture, food processing, medicine, cosmetics, and biotechnology.[2,3] Medical and health care products such as wound dressings, gauzes, *in vivo* adsorbable sutures, etc., have the highest market value among the products sold in the above-mentioned areas.[4] Hirano[5] proposed parameters such as source, appearance, viscosity or molecular weight, particle size, degree of acetylation, solubility in aqueous 0.5% acetic acid, moisture content, ash for the stan-

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Contract grant sponsor: National Science Council, Republic of China; contract grant number: NSC 86-2815-C-019-017-B.

Journal of Applied Polymer Science, Vol. 84, 193–202 (2002)

dardization of chitin and chitosan used in medical, cosmetic, standard, and industrial applications. Besides the three most commonly used parameters of molecular weight, degree of acetylation, and solubility, parameters such as heavy metal, microbial, protein, and pyrogen contents are considered to be important, and need to be specified for medical usage. The regeneration process is a frequently employed and practical measure to remove those unwanted contaminants[6–8] or to improve the solubility of the prepared chitin.[9,10] Seo et al.[11] reported that regenerated chitosan generally consists of more purified chitosan, containing fewer residual metal ions, and was found to be susceptible to lysozyme and to have slight immunoadjuvant activity through the activation of mouse peritoneal macrophages. Different regeneration methods such as regeneration using 5% aqueous sodium hydroxide solution at room temperature[8–10,12] or by using alkali–alcohol–water mixtures[13] have been reported. Regenerated chitosans have been used in the form of fibers, membranes, porous beads, and microspheres.[11] The functional properties of these products depend on their solubility, molecular weight, and degree of deacetylation of the regenerated chitosan used. The effect of using different regeneration methods and/or conditions on the physicochemical properties such as solubility, molecular weight and its distribution, and dynamic properties of the regenerated chitosan gel, e.g., creep compliance, have not been explored systematically. The objective of this study was to explore the effects of using chitosans of different molecular weights and/or degrees of deacetylation and different regeneration methods and conditions on the yield, solubility, molecular weights, and creep compliance of the regenerated chitosans.

MATERIALS AND METHODS

Chitin and Chitosan Preparation

Chitin was prepared from shrimp (*Solenocera prominentis*) waste by the modified method of Stanley et al.[14] 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 40–60 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; then it was washed with water until neutral. The alkali-treated powder 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 obtain a white chitin powder. Chitin powder was alkali treated (50% NaOH) at 100°C for 1, 2, and 3 h to obtain chitosans of different degrees of deacetylation. These were washed and dried at 50°C to obtain the final products.

Chitosan–Acetic Acid Solution Preparation

Chitosans with different degrees of deacetylation were dissolved in 5% acetic acid to make 1% solutions, then filtered through filter paper (Toyo No. 1, 90 mm, Toyo Roshi Kaisha, Japan) to remove insoluble materials.

Preparation of Regenerated Chitosans

Recovery by Alkali Solution

Ten milliters of 1% chitosan–acetic acid solutions was added to 80, 40, 20, and 10 mL of 1, 2, 4, and 8 N NaOH solutions, respectively, to precipitate the chitosan.[8,12] The precipitates were collected with a 325-mesh sieve and washed with water until neutral. The precipitates were dried to obtain the product.

Recovery by Organic Solvents

Recovery by Acetone. Ten milliters of 1% chitosan–acetic acid solution was added to 20, 40, 80, 160, and 320 mL of acetone to precipitate the chitosan. The precipitates were collected with filter paper (Toyo Roshi Kaisha Ltd., No. 1, 90 mm, Japan), and dried to obtain the product.[9]

Recovery by Alcohol. Twenty, 40, 80, 160, and 320 mL of alcohol was added to 10 mL of 1% chitosan–acetic acid solution to precipitate the chitosan. Then the chitosans were collected and dried as described in the previous section.[11]

Recovery by Alkali–Alcohol–Water Mixture

The amounts of 10, 20, 40, and 80 mL of an alkali–alcohol–water mixture (NaOH:alcohol:water = 1:3:6) was added to 10 mL of 1% chitosan–acetic acid solution to precipitate the chitosan. Then the chitosans were collected and dried as described in the section above.[11,13]

Recovery by Dialysis

A 1% chitosan–acetic acid solution was dialyzed with a dialysis bag (Spectra/Por MWCO: 3500, Spectrum Medical Industries, USA) to remove salts and ions. The residues were collected and lyophilized to obtain the products.[15]

Molecular Weight Determination of Chitosan and Regenerated Chitosans

The molecular weights of the prepared chitosan and obtained regenerated chitosans were determined with high performance liquid chromatography (HLPC) by the methods of Chen et al.[16] and Yomota et al.[17] 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. A 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 were analyzed by Chem-Lab software (Scientific Information Service Corp., Taiwan). Chitosans with known molecular weights (determined by a light-scattering method) were used as the reference. A standard curve of elution volume and molecular weight was established. The weight-averaged molecular weights of the samples were calculated from the standard curve with Chem-Lab software.

Degree of Deacetylation Determination

The degree of deacetylation of the prepared chitosan and obtained regenerated chitosans were determined by Fourier transform infrared (FTIR) methods.[18] Chitosan powder was mixed with KBr (1:100) and pressed into a pellet. The absorbencies of amide 1 (1655 cm^{-1}) and the hydroxyl band (3450 cm^{-1}) were measured using a Bio-Rad FTS-155 infrared spectrophotometer. The percentage of the amine group's acetylation in a sample is given by $(A_{1655}/A_{3450}) \times 115$. Here, A_{1655} and A_{3450} are the absorbencies at 1650and 3450 cm^{-1}, respectively.

Solubility Test

The solubilities of the prepared chitosan and obtained regenerated chitosans were determined by the method of Yalpani and Hall.[19] First 0.05 g of chitosan was added to 5 mL of distilled water (pH ca. 6.25) (Millipore, MiniQ UV Plus, USA) and stirred at room temperature for 3 h. The solution was passed through filter paper, the filter paper was weighed, and the solubility calculated from the weight gain of the filter paper.

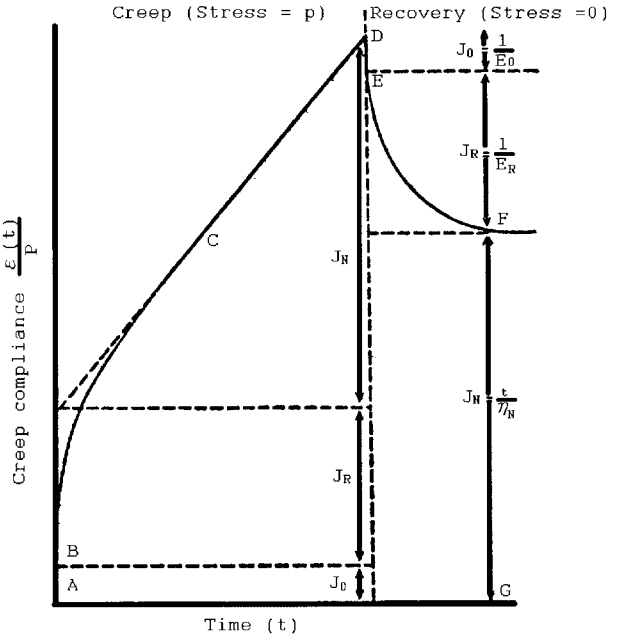

Figure 1 Creep compliance vs time curve of a viscoelastic material.

Creep Compliance Test

Creep behavior under compression was analyzed using a rheometer (Rheolab MC 120, Physica Mebtechnik GmbH, Stuttgart, Germany). A gel of the regenerated chitosan was compressed by a plate and plate cell with a gap of 2 mm, at a crosshead speed of 420 mm/s under a constant force of 1500 dyne/cm^2. Based on preliminary tests to ensure measurements were made within the linear range, creep and recovery were measured for 50-s intervals each at 20°C.

Analyses

A type creep curve for regenerated chitosan gel is shown in Figure 1. This curve was analyzed by a four-element Burger's body consisting of a Kelvin model in series with a Maxiwell model. The compression portion of the curve could be divided into three regions of instantaneous elastic compliance (AB), retarded elastic compliance (BC), and the Newtonian compliance (CD). The recovery portion of the curve could be also divided into three parts of instantaneous elastic recovery (DE), retarded

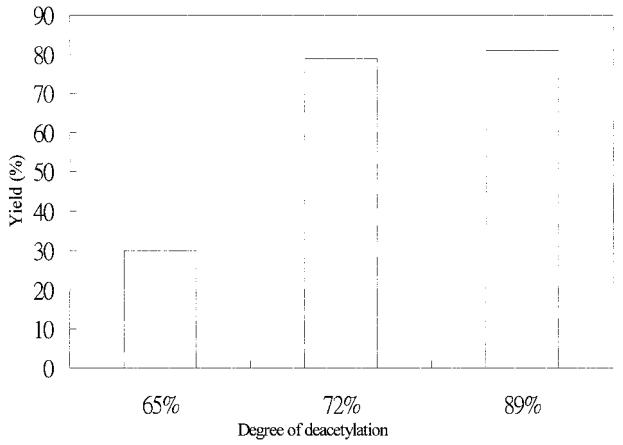

Figure 2 Effect of degree of deacetylation of chitosan used on the yield of regenerated chitosan by dialysis at 20°C.

elastic recovery (EF), and a irrecoverable deformation (FG).[20] The compliance at time t in the creep phase is

$$J(t) = J0 + Jr(t) + Jn(t) \quad (1)$$

Here, J_0 is the instantaneous elastic compliance, $J_r(t)$ is the retarded elastic compliance, and $J_n(t)$ is the Newtonian compliance. Here, $J_r(t) = J_m\ [1 - \exp(-t/\tau_m)]$, where J_m is the maximum viscoelastic compliance and τ_m is the mean retardation time, while $J_n(t)$ is equal to t/η_N, where η_N is the Newtonian viscosity. The compliance at time t in the recovery phase is

$$J(t) = J\ \max - J0 - Jr(t) \quad (2)$$

Here, $J_{\max}$ is the maximum creep compliance. J_0 is the instantaneous elastic compliance and $J_r(t)$ is the retarded elastic compliance in the recovery phase. Data were analyzed using Universal Software (US200 version 1.60, Physica) developed for creep analysis to calculate the instantaneous compliance, maximum viscoelastic compliance, mean retardation time, and the Newtonian viscosity of the creep phase and the instantaneous compliance, maximum viscoelastic compliance, mean retardation time, and, steady state compliance (J_{e0}) (irrecoverable deformation), to elucidate the elastic and recovery characteristic of the regenerated chitosan gel.[20–22]

RESULTS AND DISCUSSION

Effect of Methods and Conditions on the Yields of Regenerated Chitosans

Regeneration by Dialysis

Results in Figure 2 show that the higher the degree of deacetylation (DD) of chitosan used, the higher the yield of the regenerated chitosan obtained. Yields of 72 and 89% DD chitosans were around 80% and did not significantly differ, whereas the yield of 65% DD chitosan was around 30% only. For chitosans of the same M_w but different DDs, higher DD chitosans contain more amino groups and should consume more alkali in the precipitation process. Furthermore, the solubility of lower-DD or higher-M_w chitosans are lower than the higher-DD or lower-M_w ones, respectively.[10,23–25] Therefore, lower-DD chitosan should be more easily precipitated than those higher-DD chitosans. However, results in Figure 1 show the contrary; this may be attributed to the concentrations of chitosan used prior to dialysis being different. As described in the chitosan–acetic acid solution preparation in Materials and Methods, the insoluble materials were filtered and discarded prior to the solution proceeding to the regeneration procedure. This may be the reason that, after dialysis, the yields of higher-DD chitosan solutions were higher. Yields of 72 and 89% DD chitosans did not significantly differ. This may be due to the solubility of 72% DD chitosan and 89% DD chitosans in acetic acid being similar.

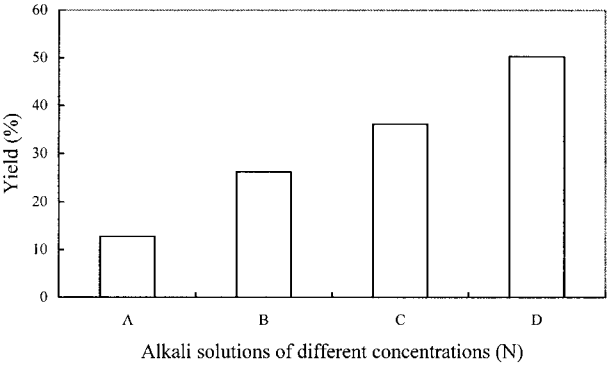

Figure 3 Effect of using the same amount of chitosan but different concentrations of alkali precipitants on the yield of regenerated 65% DD chitosans at 20°C [10 mL of a 1% chitosan–acetic acid solution was added to (A) 1 N NaOH, 80 mL; (B) 2 N NaOH, 40 mL; (C) 4 N NaOH, 20 mL; or (D) 8 N NaOH, 10 mL].

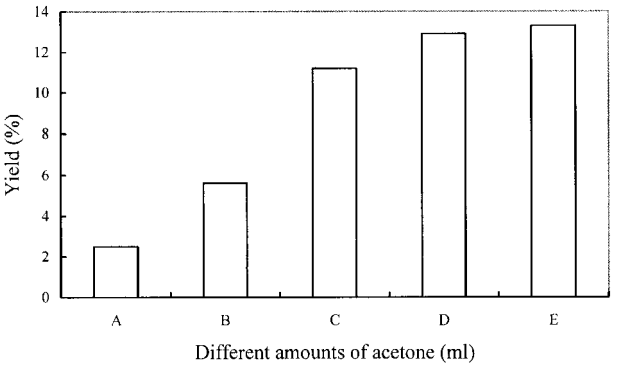

Figure 4 Effect of using different amounts of the precipitant acetone on the yield of regenerated 65% DD chitosans at 20°C [10 mL of a 1% chitosan–acetic acid solution was added to (A) 20 mL, (B) 40 mL, (C) 80 mL, (D) 160 mL, or (E) 320 mL acetone].

Recovery with Different Concentrations of Alkali Solution

Results in Figure 3 show that the yield of regenerated chitosan increased from about 10% for those using a 1 N NaOH solution to near 50% for those using an 8 N NaOH solution. This may be due to the alkali solution increasing the solution pH. The hydroxide ions screen the protonated amino groups of chitosan molecules, thus decreasing the third electroviscous effect. Because the pKi of chitosan is around pH 6.3 ~ 7.0,[26,27] decreases in the inter- and intramolecular repulsion forces in turn increase the intermolecular aggregations and precipitation events. Although the total amount of alkali used was equal when using different concentration alkali solutions, the effectiveness of higher concentrations is greater. This may be attributed to the chemical potential of higher alkali concentration solutions being higher than those of lower concentration ones.

Recovery with Different Amounts of Organic Solvents

Results in Figure 4 show that the yield of regenerated chitosan increased linearly with increasing amounts of acetone used between 20 and 160 mL. Increases in yield reached a plateau at volumes of acetone used larger than 160 mL. The highest yield was only 13% obtained by using 320 mL acetone. Yields of regenerated chitosan obtained by using alcohol were very poor. Using a volume of alcohol as high as 32 times that of the chitosan–acetic acid solution did not result in significantly greater yields (data not shown). This may be attributed to the mechanism of precipitation of the regenerated chitosan by acetone is that acetone has to compete with water molecules of the chitosan. Decreasing numbers of water molecules in the chitosan will facilitate the intermolecular aggregation of chitosan molecules and lead to precipitation. This process needs larger volumes of acetone to deplete the water molecules from the chitosan molecules. Therefore, the effectiveness of using organic solvents is lower than that of using alkali solution; furthermore, organic solvents are more expensive and more hazardous to the environments than are alkali solutions.

Recovery with the Alkali–Alcohol–Water Mixture

Results in Figure 5 show that the yield of regenerated chitosan increased linearly from near 14 to around 25% with increasing volume of the alkali–alcohol mixture used from 10 to 80 mL. However, the highest yield was only around 25% with 80 mL of the alkali–alcohol–water mixture used. In comparing the results shown in Figure 3, the highest yield was around 50%. The total amount of alkali used in the alkali–alcohol–water mixture ranged from 1 to 8 g compared to 3.2 g for those alkali solutions. The yield of regenerated chitosan obtained by using 80 mL of the mixture was close to that of using a 2 N alkali solution. However, the concentration of alkali in the 80 mL mixture was 2.5 N NaOH. Therefore, the effectiveness of alkali in alkali–alcohol–water mixture was lower than that in the alkali solution. The effectiveness of alcohol in the alkali–alcohol–water mixture on the precipitation of chitosan was not significant.

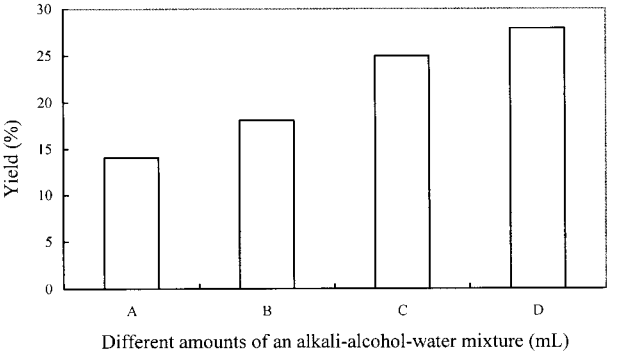

Figure 5 Effect of using different amounts of the precipitant of an alkali–alcohol–water mixture on the yield of regenerated 65% DD chitosan at 20°C [10 mL of a 1% chitosan–acetic acid solution was added to (A) 10 mL, (B) 20 mL, (C) 40 mL, or (D) 80 mL mixture of alkali:alcohol:water = 1:3:6].

Table I Effect of Different Kinds and Concentrations of Precipitant on the Solubility of Regenerated Chitosans

Regeneration Methods, Kinds and Concentrations of Precipitants	Regenerated Chitosans		
	65% DD	72% DD	89% DD
Original chitosan	0.0105*cx*[a,b]	0.0051*bx*	0.0089*cx*
Dialysis[c]	0.0160*bx*	0.0133*ax*	0.067*cx*
1 N NaOH[d]	0.0241*ax*	0.0072*by*	0.0188*ax*
2 N NaOH[d]	0.0251*ax*	0.0117*abz*	0.0197*ay*
4 N NaOH[d]	0.0227*ax*	0.0138*ay*	0.0156*by*
8 N NaOH[d]	0.0262*ax*	0.0108*aby*	0.0154*by*
Alkali–alcohol–water mixture[e]	0.0259*ax*	0.0130*az*	0.0169*aby*

[a] *a–c:* Means with the same lower case italic letter within the same column do not differ significantly ($p > 0.05$ by Duncan's multiple range test).

[b] *x–z:* Means with the same lower case italic letter within the same row do not differ significantly ($p > 0.05$ by Duncan's multiple range test).

[c] Regenerated by dialysis of a 1% chitosan–acetic acid solution with distilled water through a dialysis bag (Spectra/Por MWCO: 3500, Spectrum Medical Industries, USA).

[d] Using the same amount but different concentrations of alkali, e.g., 80, 40, 20, and 10 mL of 1, 2, 4, and 8 N alkali solutions, respectively, were added to 10 mL of a 1% chitosan–acetic acid solution to precipitate the chitosan.

[e] Eighty milliters of an alkali–alcohol–water mixture (alkali : alcohol : water = 1 : 3 : 6) was added to 10 mL of a 1% chitosan–acetic acid solution to precipitate the chitosan.

The precipitation was totally influenced by the alkali.

Solubility of Original and Regenerated Chitosans

Table I shows that the original chitosan samples with DDs between 65 and 89% have similar solubilities at about 0.0051 to 0.0105 in distilled water, which are very low values. Solubility of those regenerated chitosans increased slightly. The solubility of regenerated chitosans prepared by dialysis treatment did not increase significantly and were between 0.016 and 0.067. Solubilities of regenerated chitosans obtained by precipitation with different concentrations of alkali solution were in the range of 0.0108–0.0262. Those precipitated from the alkali–alcohol–water mixture were in the range of 0.0130–0.0259. However, different regeneration methods and conditions used did not result in significant differences. This may be became those regeneration treatments did not change the structure nor the molecular weight significantly of the precipitated chitosans. The regenerated chitosans are those insoluble in the recovery medium, so therefore the solubilities of those products will not differ significantly among each other. However, the regeneration procedure may result in precipitating different molecular weight chitosans. Therefore, the molecular weights of regenerated chitosans were lower than the original chitosan samples as shown in Table II.

Molecular Weights of Original and Regenerated Chitosans

Table II shows the effect of regenerated methods and conditions used on the changes of molecular weight of regenerated chitosans. Molecular weights of three original chitosan samples did not differ significantly and are exceptionally high, and in the range of $(2.32 \sim 2.53) \times 10^7$ Da. This result is inconsistent with that reported in much of the literature.[25,28,29] Molecular weights of chitosans obtained from alkali-deacetylated chitin should decrease with increasing degree of deacetylation. In general, higher concentrations of alkali solutions, elevated reaction temperatures, and longer reaction times used in the alkali deacetylation reaction will result in higher deacetylated and lower molecular weight products. Therefore, the above results may be due to the occurrence of aggregations of chitosan molecules. Chitosan is prone to aggregate in solution[30–32] resulted in anomalous low value of the Mark–Houwink exponent a in turn having higher molecular weights detected by the high performance liquid chromatography (HPLC) method. Molecular weights of regenerated chitosans were lower than those of the original chitosans. Molec-

Table II Effect of Regeneration Method, and Kinds and Concentrations of Precipitants, Used on Changes in Molecular Weight of Regenerated Chitosans

Regeneration Methods, Kinds and Concentrations of Precipitants	65% DD	72% DD	89% DD
Original chitosan	2.53E+7*ax*[a,b]	2.37E+7*ay*	2.32E+7*ay*
Dialysis[c]	1.92E+7*dx*	2.04E+7*bx*	2.10E+7*bx*
1 N NaOH[d]	2.37E+7*bx*	2.04E+7*by*	1.68E+7*dz*
2 N NaOH[d]	2.38E+7*b*	—	—
4 N NaOH[d]	2.21E+7*cx*	2.03E+7*by*	1.56E+7*ez*
8 N NaOH[d]	2.24E+7*cx*	2.01E+7*by*	1.77E+7*cz*
Alkali-alcohol-water mixture[e]	2.16E+7*cx*	—	1.81E+7*cy*

[a] *a–e:* Means with the same lower case italic letter within the same column do not differ significantly ($p > 0.05$ by Duncan's multiple range test).
[b] *x–z:* Means with the same lower case italic letter within the same row do not differ significantly ($p > 0.05$ by Duncan's multiple range test).
[c] Same as in Table III.
[d] Same as in Table II, footnote d.
[e] Same as in Table II, footnote e.

ular weights of regenerated 65% DD chitosans obtained by precipitation with alkali solution decreased with increasing concentration of the recovery alkali solution. The regenerated 65% DD chitosans obtained with the alkali–alcohol–water mixture have similar molecular weights to those obtained by using 4 or 8 N NaOH solutions. After dialysis, the molecular weight of the regenerated 65% DD chitosan decreased to 1.92×10^7 Da and was slightly lower than those obtained by using 4 or 8 N NaOH solutions. Molecular weights of regenerated 72 or 89% DD chitosans obtained by using different concentrations of alkali solutions also showed decreases. Using the same regeneration conditions, the magnitudes of decreases in molecular weight are proportional to the DD of original chitosans used. For example, M_ws of 65, 72, and 89% DD regenerated chitosans are 2.37, 2.04, and 1.68×10^7 Da, respectively, for those regenerated with a 1 N NaOH solution. The molecular weights of regenerated chitosan obtained by dialysis, by precipitation with different concentrations of alkali solution, or with the alkali–alcohol–water mixture decreased with increasing DD of the original chitosan used. This may be due to molecular weight of regenerated chitosan merely reflecting the differences of the original chitosan samples used. Usage of higher molecular weight chitosan should produce higher molecular weight products after regeneration treatment. Since the solubility of 65% DD chitosan is low, the yield was lower (Fig. 2). However, the M_ws of those soluble constituents were higher than that of 72% DD chitosan, and in turn were higher than that of 89% DD chitosan.

Creep Compliance of Regenerated Chitosans Obtained by Different Media

Figures 6–8 show the creep compliances of the gel formed by regenerated 65, 72, and 89% DD chitosan, respectively, obtained by using different concentrations of alkali solution or the alkali–alcohol–water mixture. Instantaneous compliances, maximum viscoelastic compliances, mean retardation times, and steady static compliance (irrecoverable deformation) of regenerated hydrogels of 65% DD chitosans were calculated from Figure 6 and listed in Table III. The instantaneous creep compliance of the gel formed by re-

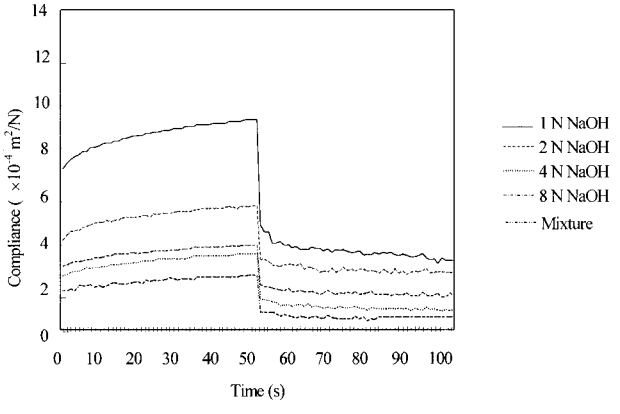

Figure 6 Creep-compliance curves of 65% regenerated chitosan measured at $\tau = 1500$ dyne/cm^2 for 100 s at 20°C. Regenerated chitosans were precipitated by added the same amount but different concentrations of 1, 2, 4, and 8 N NaOH, or by using 80 mL of an alkali–alcohol–water mixture (alkali:alcohol:water = 1:3:6) to 10 mL of a 1% chitosan–acetic acid solution.

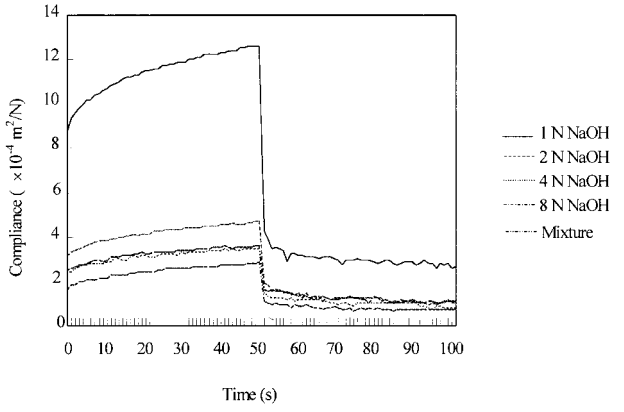

Figure 7 Creep-compliance curves of 72% regenerated chitosan measured at $\tau = 1500$ dyne/cm^2 for 100 s at 20°C. Regenerated chitosan was precipitated by added the same amount but different concentrations of 1, 2, 4, and 8 N NaOH, or by using 80 mL of an alkali–alcohol–water mixture (alkali:alcohol:water = 1:3:6) of a 1% chitosan–acetic acid solution.

generated chitosans precipitated by higher-concentration alkali solutions was lower than those obtained with lower-concentration alkali solutions. The values were 4.0057, 2.0400, 1.4089, and 1.5568 $\times$ 10^{-4} Pa^{-1}, for those recovery with 1, 2, 4, and 8 N NaOH, respectively. This may be due to the fact that concentrations of the gels formed by regenerated chitosans precipitated from lower-concentration alkali solutions are lower than those formed by regenerated chitosan precipitated from higher-concentration alkali solutions. Because, the yield of regenerated chitosan increased with the concentration of alkali solution used (Fig. 3). The results imply that the gels of regenerated chitosans precipitated by higher alkali concentrations were firmer than those precipitated by lower alkali concentrations. Mean retardation time of the gels formed by regenerated chitosan precipitated by higher-concentration alkali solutions were longer than those obtained with lower-concentration ones. The values were 7.1724, 5.7644, 9.9755, and 10.989 s for those recoveries with 1, 2, 4, and 8 N NaOH, respectively. The longer retardation time means slower deformation or firmer gel. Shorter retardation time of hydrogel regenerated by low-concentration alkali solutions corresponded to creep-compliance curves in which rapidly increase compliance were clearly observed in the retardation part. The steady static compliance (irrecoverable deformation) was larger for those gels formed by regenerated chitosan obtained by using lower alkali concentration solutions. The values were 4.2384, 1.9801, 1.6635, and 1.4995 $\times$ 10^{-4} Pa^{-1} for those recovery by 1 N, 2 N, 4 N, and 8 N NaOH, respectively. Creep compliances of the gel of regenerated chitosans precipitated by using 1 N NaOH increased with increasing DD of the regenerated chitosans. (Figs. 6–8). This may be

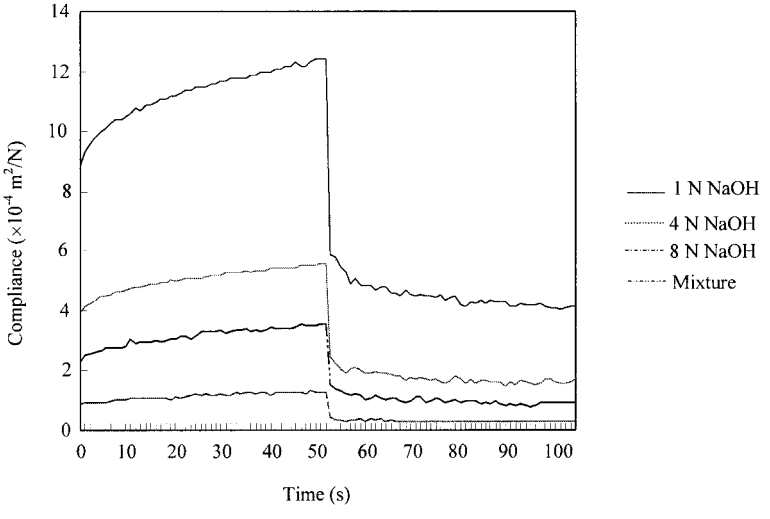

Figure 8 Creep-compliance curves of 89% regenerated chitosan measured at $\tau = 1500$ dyne/cm^2 for 100 s at 20°C. Regenerated chitosan was precipitated by adding the same amount but different concentrations of 1, 2, 4, and 8 N NaOH, or by using 80 mL of an alkali–alcohol–water mixture (alkali:alcohol:water = 1:3:6) to 10 mL of a 1% chitosan–acetic acid solution.

Table III Instantaneous Compliances, Maximum Viscoelastic Compliances, Mean Retardation Times, and Steady Static Compliance of Hydrogels of Regenerated 65% DD Chitosan

	1 N NaOH	2 N NaOH	4 N NaOH	8 N NaOH	Mixture
Creep phase					
Instantaneous compliance (J_0, 1/Pa)	4.0057E-4	2.04E-4	1.4089E-4	1.5568E-4	1.0545E-4
Maximum viscoelastic compliance (J_m, i/pa)	8.3027E-5	5.5784E-5	3.5144E-5	3.7967E-5	1.7381E-5
Mean retardation time (s)	7.1724	5.7644	9.9755	10.989	5.7745
Zero shear viscosity (Pa · s)	6.647E6	9.2621E5	1.3956E6	1.7800E6	1.3988E6
Recovery phase					
Instantaneous compliance (J_0, 1/pa)	3.4441E-4	1.5769E-4	1.3666E-4	1.2021E-4	1.0096E-4
Maximum viscoelastic compliance (J_m, 1/pa)	7.9426E-5	4.0322E-5	2.9694E-5	2.9736E-5	2.7009E-5
Mean retardation time (s)	23.067	11.009	11.868	12.821	4.743
Steady state compliance (J_{e0}, 1/pa)	4.2384E-4	1.9801E-4	1.6635E-4	1.4995E-4	1.2797E-4

attributed mainly to the gels formed by different molecular weights (Table II) and/or partially to different concentrations of regenerated chitosans. Gels formed by regenerated chitosan obtained by using the alkali–alcohol–water mixture have similar creep compliances and steady state compliance as those formed by regenerated chitosan precipitated with 4 or 8 N alkali solutions. The instantaneous compliance and steady static compliance were 1.0545 and 1.2797 $\times 10^{-4}$ Pa^{-1} ,respectively. This may be due to the molecular weight of those regenerated chitosans being similar (Table II).

CONCLUSION

Yield of regenerated chitosan obtained by dialysis was the highest among methods explored. The higher the alkali concentrations, the higher the yield that gels regenerated. Creep compliance of regenerated chitosan gels obtained from 65% DD chitosan was lower than that of either 72or 89% DD chitosans. Of the same DD chitosan, creep compliances of regenerated chitosan gels obtained by using a higher concentration of alkali solution were lower than that obtained by using lower concentration ones. Hydrogels regenerated from different DD chitosans and/or different recovery mediums have different structure and tactile properties. Therefore, they can be used as wound dressing suited to different applications.

The authors wish to express their appreciation for financial support from National Science Council, Republic of China (Project No. NSC 86-2815-C-019-017-B).

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