Chitosan Hydrogels: Crosslink Kinetics and Gel Properties

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INTRODUCTION

Chitin is the second most abundant natural biopolymer on earth and is composed of β(1→4)-linked 2-acetamido-2-deoxy-β-D-glucose (N-acetylglucosamine). The principle derivative of chitin, chitosan, is obtained by N-deacetylation, to a varying extent that is characterized by the degree of deacetylation, and is consequently a copolymer of N-acetylglucosamine and glucosamine (Figure 1a). Chitin and its derivatives have found wide application in a variety of areas such as: medicine, pharmaceutics, paper production, textiles, metal chelation, food additives, antimicrobial agents, adhesives, and other industrial applications. We recently prepared a water-soluble, blocked diisocyanate as crosslinking agents for network formation.[1] This should ease the processability of chitosan gel formations, which is typically hindered by solubility issues. With greater control over crosslink efficiency, physical, mechanical, and biological properties of the chitosan gels can be tailored for a given application.

A detailed understanding of the network structures and properties is required if chitosan based hydrogels are to realize their full potential. Here, we study the cure kinetics and network properties of the network formation processes by investigating the gel time (onset of gelation) and the gel modulus (elastic modulus of the cured networks). This investigation provides a basis from which to explore cell activity on scaffolds with gradient material properties.

EXPERIMENTAL

Deacetylation of Chitosan. Seacure chitosan (Vanson-Halosource*) was further deacetylated[2] by suspending 200 g of the commercial preparation in 20 % NaOH (2.5 L) at 60 °C for 2 h under a nitrogen atmosphere. After a water wash (20 L), the alkali treatment was repeated once. The remaining solids were then washed with water, until the pH matched that of the wash water, and were then dissolved in 2 % acetic acid (4 L). Acid-insoluble material was removed by filtration and the chitosan was isolated by precipitation into 1 N NaOH (6 L). After extensive dialysis against water (5 d), the chitosan was neutralized by addition of dry ice and decolorized by refluxing in acetone for 2 h under a nitrogen atmosphere. Dried solids were pulverized in a mill to 200 μm sieve pass. The final product (122 g) was obtained after drying *in vacuo* at 50 °C for 48 h.

Synthesis of hexamethylene-1,6-di-(aminocarboxysulfonate) (Figure 1 b). In a 100 mL round-bottom flask containing a magnetic stir bar, 6.73 g (40 mmol) hexamethylene diisocyanate was added to 8.36 g $Na_2S_2O_5$ (44 mmol) dissolved in 15.53 mL H_2O and was stirred for 20 h at room temperature. The product was precipitated in acetone and dried *in vacuo*. The polymer was removed by dissolving the product in water (30 mL) followed by filtration. The product was isolated from the filtrate by precipitation in acetone and dried *in vacuo*, resulting in a white powder with a 75 % yield.

Rheology sample preparation and measurements. Chitosan solution (10 % chitosan by mass fraction, 5 % by mass fraction acetic acid) and the calculated amount of crosslinker were weighed into a clean vial. Additional water was added to adjust the chitosan concentration to 5 % by mass fraction. The reagents were mixed thoroughly, and then centrifuged to remove air bubbles. Samples were stored in a refrigerator and were generally used within three days of preparation. Rheological measurements were performed on a Rheometric Scientific ARES instrument with a parallel plate geometry (25 mm diameter). Solvent (water) evaporation was minimized by covering the edges of the sample in a pool of low viscosity silicone oil. In general, no water evaporation was detected for at least 15 h with adequate oil coverage. Duplicate experiments showed excellent reproducibility with relative standard uncertainty of 3 %.

RESULTS AND DISCUSSION

The chitosan prepared for these studies was found to have a degree of deacetylation of 88.6 % (^{1}H NMR),[1] a viscosity-average molecular weight of 220,000 g/mol, and to be readily soluble in dilute acetic acid solutions. These values correspond to an amine concentration of 5.3 mmol/g of chitosan and a molecular formula given by Figure 1a, where x = 1203 and y = 154.

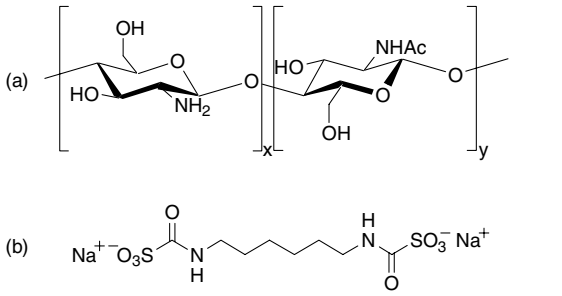

Figure 1. Structure of chitin (x = 0) and chitosan (a), and hexamethylene-1,6-di-(aminocarboxysulfonate) (b).

We first studied the effect of temperature, between 60 °C and 90 °C, on the cure kinetics and the resultant gel modulus using dynamic time sweep experiments. A small strain amplitude (2 %) was applied at a frequency of 1 rad/s to ensure that the experimental conditions would not interfere with the gelation process. The ratio of aminocarboxysulfonate on the crosslinker to amine on chitosan was maintained at 20 %. Figure 2 shows an example of the storage modulus (G') and loss modulus (G") measured as a function of cure time at 70 °C. The data, collected before gelation, are near the sensitivity limit for the transducer and are therefore noisy. Both G' and G" increased above the gelation and eventually reached plateau at high crosslink conversions.

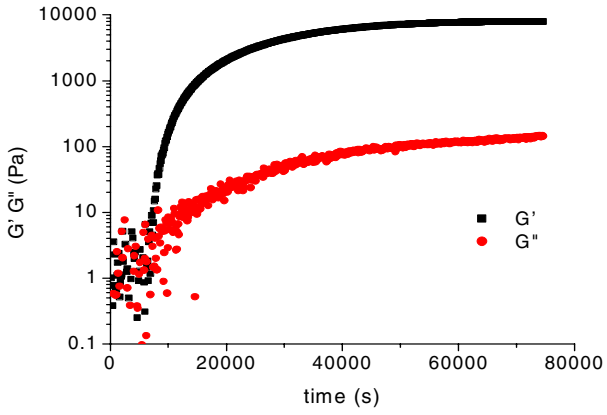

Figure 2. Dynamic time sweep of chitosan with 20 % crosslinker measured at 70 °C.

* *Certain commercial materials and equipment are identified in this paper in order to specify adequately the experimental procedure. In no case does such identification imply recommendation by the National Institute of Standards and Technology nor does it imply that the material or equipment identified is necessarily the best available for this purpose.*

Dynamic frequency sweeps were measured following each dynamic time sweep to assess the degree of crosslinking. Figure 3 shows the G' and G" for a chitosan hydrogel cured with 20 % crosslinker at 70 °C (the cure profile shown in Figure 2) where both G' and G" were nearly independent of the frequency in the frequency region probed. This verifies solid-like behavior in the cured systems and suggests that a high degree of reaction conversion was obtained. All networks cured at various temperatures showed similar frequency sweep profiles.

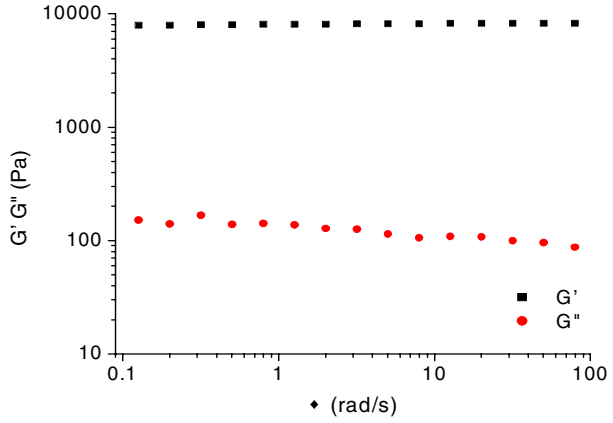

Figure 3. Dynamic frequency sweep of fully cured chitosan cured with 20 % crosslinker at 70 °C.

From the dynamic time sweep experiments and the subsequent dynamic frequency sweeps, we determined both the gel time and the gel modulus (Table 1). The gel time is defined as the time at which G' intersects G" from the dynamic time sweep measurements, and the gel modulus is the modulus observed at 1 rad/s from the corresponding dynamic frequency sweeps. As expected, the gel time decreased as the cure temperature increased since the reaction proceeded more rapidly at elevated temperatures. The gel moduli of networks crosslinked at different temperatures (between 80 °C and 90 °C) were comparable. The gel modulus cured at lower temperatures could not be determined accurately due to the extremely long time required to reach high crosslink conversions.

Table 1. Gel time measured at various temperatures for chitosan cured with 20 % crosslinker

T (°C)	Gel Time (s)	Gel Modulus (Pa)
60	18000 ± 600	-
70	6800 ± 200	-
80	2150 ± 100	9700 ± 300
85	1350 ± 50	9600 ± 300
90	900 ± 50	9700 ± 300

An Arrhenius analysis was used to calculate the apparent activation energy for the gel forming processes. Figure 4 shows a semi-logarithmic plot of the gel time as a function of inverse reaction temperature, which exhibits a linear relationship. An apparent activation energy of 10.4 kJ/mol was calculated using the slope of the plot.

The effect of crosslinker composition was also investigated. To vary the degree of crosslinking, the blocked diisocyanate (Figure 1b) was added to acidic chitosan solutions (5 % by mass fraction) with isocyanate to amine ratios (NCO/NH_2) of (0, 10, 20, 30, 40 and 50) %. Figure 5 shows the G' observed from dynamic time sweeps for chitosan cured with various amount of crosslinker. Interestingly, the modulus for the chitosan solution without crosslinker increased after exposure to elevated temperatures. However, the modulus of the pure chitosan was significantly lower than the chemically crosslinked networks. As expected, gelation occurred more rapidly with increased crosslinker. The gel modulus also increased with increased crosslinker due to the formation of more highly crosslinked networks.

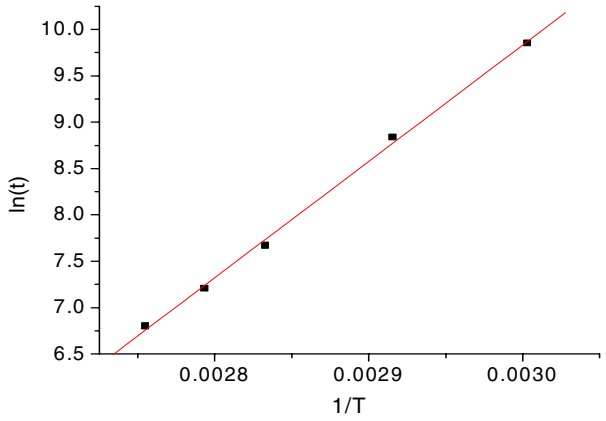

Figure 4. Arrhenius plot for chitosan cured with 20 % crosslinker, the line is a linear fit of the data points.

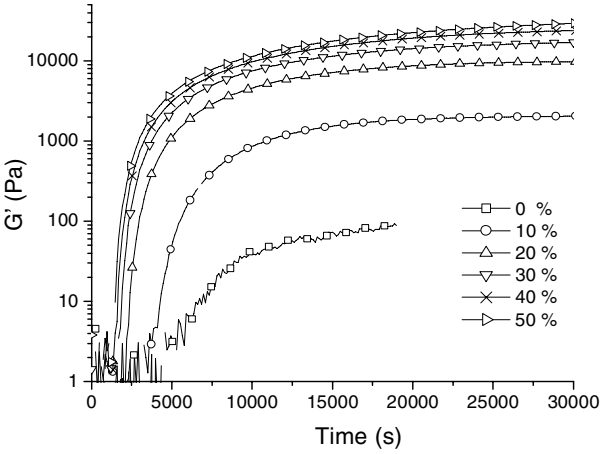

Figure 5. G' of chitosan crosslinked with various amount of crosslinker at 80 °C.

Cure kinetics and network properties of hydrogels prepared by the reaction of chitosan and a water soluble blocked diisocyanate have been measured using rheometry. The reactions follow the Arrhenius behavior from which apparent activation energies can be determined. The network properties can be tailored by adjusting the ratio of chitosan to crosslinker. We will utilize this capability to engineer tissue culture scaffolds with gradient network properties.

ACKNOWLEDGEMENTS

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