Degradation of Chitosan and Chemically Modified Chitosan by Viscosity Measurements

Sunil A. Agnihotri, Vaibhav D. Kulkarni, Anandrao R. Kulkarni, Tejraj M. Aminabhavi

Drug Delivery Division, Center of Excellence in Polymer Science, Karnatak University, Dharwad 580 003, India

Received 14 January 2006; accepted 16 April 2006
DOI 10.1002/app.24663
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Chitosan and chitosan-*grafted*-acrylamide were subjected to degradation in the presence and absence of a degrading agent at 37°C by measuring the viscosity of polymer solutions. Extracts from the fresh rat droppings were used as the degrading agent to simulate the environmental degrading conditions. Results of this study indicated that the concentrations of acetic acid in solution and chemical modifications of chitosan affected the degradation both in the presence as well as in the absence of a degrading agent. Reduction in viscosity was used to study the degradation. Chitosan was stable up to sixteen days of immersion in acetic acid without the degrading agent, but it readily underwent degradation in the presence of a degrading agent. Chitosan-*grafted*-acrylamide also followed the same trend, but the extent of reduction in viscosity was much less than pure chitosan, indicating that the chemical modification has improved the stability of the polymer. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 3255–3258, 2006

Key words: chitosan; degradation; chemical modification; viscosity

INTRODUCTION

Chitosan is a biopolymer obtained by deacetylation of chitin. Chitin is the second most abundant biopolymer available in nature, which is found in the exoskeleton of crustaceans, fungi, etc.[1] Chitosan has been used as a matrix material in drug delivery,[2] preservation of food products, artificial tissues, etc. In these applications, its degradation plays an important role. Chitin and chitosan represent the long-chain polymers having molecular weight up to several million Daltons. Commercially available chitosan has an average molecular weight ranging between 3800 and 20,00,000 Daltons and is 66–95% deacetylated. Chitosan has glucosamine units, which undergoes degradation similar to other carbohydrates like starch. Chitosan is known to degrade in human serum *in vitro*.[3] Lysozymes of human milk and hen egg white were shown to degrade chitosan.[4] Lipase from the roots of R. japonicus could also degrade chitosan to give oligomers. Papain degrades chitosan, the extent of which depends upon the degree of acetylation.[5] Highly acetylated chitosan is more susceptible to degradation than lower acetylated chitosan. Degradation products obtained are usually oligomeric units, some of which have the potential biomedical applications.[6]

Degradation of chitosan by rat cecal and colonic contents was evaluated and compared with the commercially available enzymes.[7] Degradation studies on chitosan with respect to molecular weight and degree of deacetylation of chitosan have been reported.[7] Since chitosan is soluble in acidic water, concentration of acetic acid is also important while studying its degradation. In view of the importance of chitosan as a biopolymer in drug delivery research, we found it essential to evaluate the degradation of chitosan and its chemically modified derivative. Chitosan can undergo degradation by colonic bacterial enzymes, and the rat cecum and colon have been shown to have the same microbial contents as the human colon in terms of predominant bacterial species. In the present research, fresh rat droppings were employed as the potential environmental degrading agent, which is easily available and is cost effective. However, the activity of the extracted enzymes from rat droppings could not be defined in the present work. Measuring viscosity of solutions at different time intervals monitored the process of degradation. Degradation profiles of chitosan in the presence and absence of a degrading agent was evaluated. Effect of chemical modification on degradation profile of chitosan was also investigated.

This paper is CEPS communication # 24.

Correspondence to: T. M. Aminabhavi (aminabhavi@yahoo.com).

Contract grant sponsor: University Grants Commission, New Delhi, India; contract grant number: F1-41/2001/CPP-II.

EXPERIMENTAL

Synthesis of chitosan-*grafted*-polyacrylamide

A 1.5 g of chitosan was dissolved in 2% acetic acid under constant stirring. Aqueous solution of 0.75 g acrylamide was then added to chitosan solution and stirred for 1 h at 60°C. Ceric ammonium nitrate (CAN) (100 mg) was used as an initiator for the grafting reaction. Polymerization was carried out under continuous purging of nitrogen gas under constant stirring at 60°C for 5 h. The reaction mixture was cooled and a pinch of quinhydrone was added to quench the reaction. The mass obtained was precipitated in sufficient amount of acetone and the precipitate was washed with methanol : water (8 : 2) mixture to remove the homopolymer formed. The graft copolymer formed was dried under vacuum (60 mmHg) at 40°C overnight. The percentage grafting was confirmed by weight gain by the chitosan after grafting.

Preparation of polymer solutions

Chitosan and chitosan-*grafted*-polyacrylamide (CS-*g*-pAAm) solutions were prepared by dissolving a weighed quantity (0.01% w/w) of the polymer in aqueous acetic acid solutions of 1.5, 3.0, 4.5, 6.0, and 7.5 ($\times 10^{-3}$) mole fractions.

Degradation model

Degradation model consists of an aqueous extract of fresh Wistar rat droppings. About 1 g of the droppings were collected and triturated in 50 mL of water and sonicated using a probe sonicator (UP 400 s, dr. hielscher, GmbH, Germany) for about 15 min to break the particles and facilitate the extraction. The entire solution was centrifuged at 10,000 rpm speed for 15 min using a tabletop centrifuge (Jouan, MR 23i, Cedex, France) to separate the undissolved particles. Clear supernatant liquid was added (0.9%) to polymer solutions. Polymer solutions with and without the degrading agent were incubated for 16 days at 37°C in an incubator (WTB Binder, Tuttlingen, Germany).

Viscosity measurements

Viscosity (η) measurements of all the polymer solutions were done using a Schott-Gerate Viscometer (AVS 250) at 25°C. Temperature of the bath was controlled to an accuracy of ± 0.1°C read by the digital display. Viscosity measurements were done for time periods of 1, 2, 4, 8, and 16 days. Measurement of viscosity (η_0) of various concentrations of acetic acid in water was made to calculate the specific viscosity (η_{sp}) using the relation.

$$\text{Specific viscosity } (\eta_{sp}) = \frac{\eta}{\eta_0} - 1 \qquad (1)$$

RESULTS AND DISCUSSION

Graft copolymerization of chitosan with acrylamide was achieved by CAN-catalyzed free radical polymerization. The percentage grafting of 33.3 and percentage grafting efficiency of 88.9 with a percentage conversion of acrylamide up to 66.7 were achieved. Degradation studies were conducted up to 16 days. To compensate any changes in the viscosity of acetic acid solutions over the selected time period, specific viscosity (η_{sp}) was calculated instead of viscosity. Results of specific viscosity versus time of soaking of chitosan and CS-*g*-pAAm in different concentrations of acetic acid solutions without adding degrading agent are presented in Figure 1. The change in viscosity over 16 days was very minimal for both chitosan and CS-*g*-pAAm polymers, indicating their stability in solution. As the concentration of acetic acid increases, the viscosity decreased in all the cases. This is in accordance with the previous study by Kurkuri et al.,[8] wherein, it was observed that the viscosity of chitosan solution decreased as the concentration of acetic acid increased; this is attributed to an increased protonation and hence, solubilization of the polymer. However, the concentration of acetic acid helps to increase the solubility of the polymer as well as to decrease the pH of the solution.

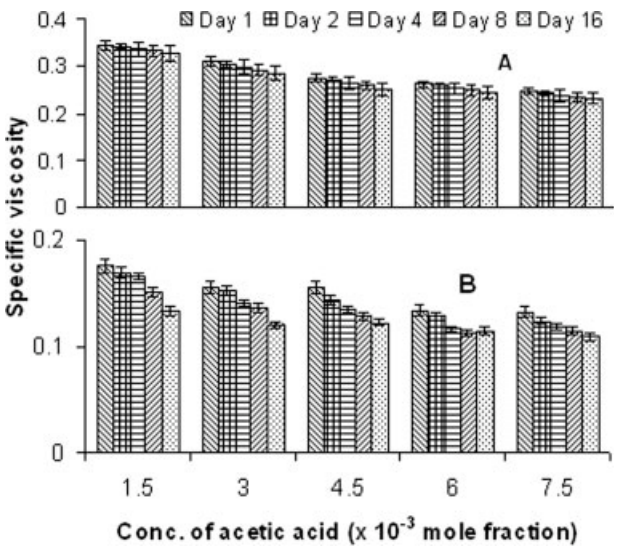

Figure 1 Degradation patterns of (A) chitosan and (B) chitosan-*grafted*-acrylamide in the absence of a degrading agent.

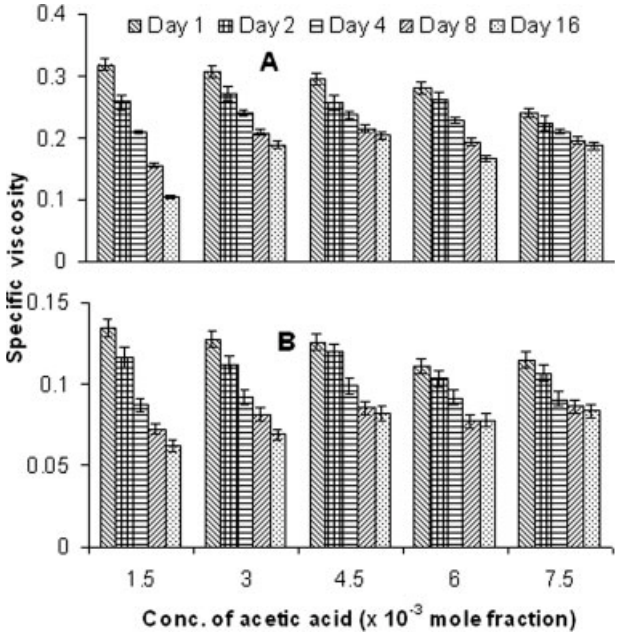

Figure 2 Degradation patterns of (A) chitosan and (B) chitosan-*grafted*-acrylamide in the presence of a degrading agent.

Results of specific viscosity versus time of immersion for chitosan and CS-*g*-pAAm polymers in various acetic acid solutions with the degrading agent are presented in Figure 2. Specific viscosity of both the polymers decreased upon exposure to the degrading agent and also, with the time of solvent immersion; however, the specific viscosity of CS-*g*-pAAm is comparatively smaller than that observed for chitosan not because of degradation, but due to its chemical modification. During grafting, chitosan chains might have broken down and many new

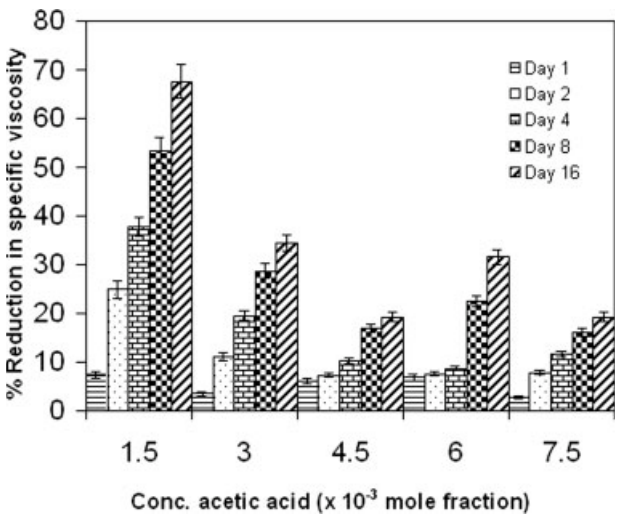

Figure 3 Percentage reduction in specific viscosity of chitosan in the presence of a degrading agent.

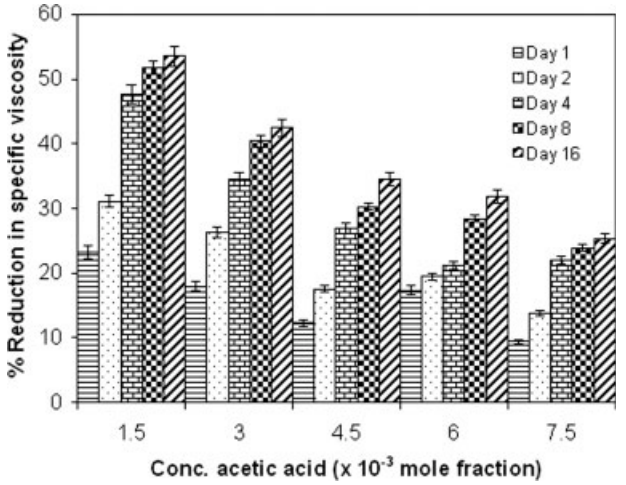

Figure 4 Percentage reduction in specific viscosity of chitosan-*grafted*-acrylamide in the presence of a degrading agent.

branches might have been formed.[9] This might have decreased the viscosity of CS-*g*-pAAm polymer as compared to neat chitosan even though the concentrations of both the polymers are same.

The degradation agent used here is microflora and the enzyme system of the rat lower intestinal tract.[7] Extent of degradation was monitored by computing percentage decrease in specific viscosity of polymer solutions. The percentage reduction in specific viscosity of chitosan and CS-*g*-pAAm polymers in the presence of a degrading agent are shown in Figures 3 and 4, respectively. A highest reduction of 68% was observed for chitosan in 1.5×10^{-3} mole fraction concentration of acetic acid, but for CS-*g*-pAAm, it was only 53%. This suggests that CS-*g*-pAAm is more stable than the neat chitosan in the selected degradation agent. The percentage reduction in specific viscosity of both the polymers decreases as the mole fraction of acetic acid increased. This can be attributed to the proliferation of microorganisms under relatively milder pH conditions (closer to neutral pH), thus resulting in an increased enzymatic activity at a particular pH.

In conclusion, chitosan and CS-*g*-pAAm polymers are quite stable in solutions of differing concentrations of acetic acid during the entire period of immersion study. Both the polymers underwent degradation readily in the presence of extracts of freshly collected rat drops (fecal matter). Activity of degrading agent was affected at higher concentrations of acetic acid, which is often used to solubilize the polymer. Grafting of acrylamide reduced the rate of degradation, and grafting renders the polymer immune to enzymatic degradation, but it might have increased the antibacterial property of chitosan. From the physiological point of view, chitosan is

readily biodegradable by enzymes as well as by microorganisms, but it degrades slowly in solutions in the present environmental degradation model chosen.

References

1. Muzarelli, R. A. A. Chitin; Pergamon Press: Elmsford, New York, 1977.
2. Agnihotri, S. A.; Mallikarjuna, N. N.; Aminabhavi, T. M. J Controlled Release 2004, 100, 5.
3. Vårum, K. M.; Myhr, M. M.; Hjerde, R. J. N.; Smidsrød, O. Carbohydr Res 1997, 299, 99.
4. Nordtveit, R. J.; Vårum, K. M.; Smidsrød, O. Carbohydr Polym 1996, 29, 163.
5. Lee, C.; Shin, S. S.; Lee, Y. C. J Food Biochem 2001, 25, 307.
6. Terbojevich, M.; Cosani, A.; Muzzarelli, R. A. A. Carbohydr Polym 1996, 29, 63.
7. Zhang, H.; Neau, S. H. Biomaterials 2002, 23, 2761.
8. Kurkuri, M. D.; Kulkarni, A. R.; Aminabhavi, T. M. J Appl Polym Sci 2002, 86, 526.
9. Kumbar, S. G.; Soppimath, K. S.; Aminabhavi, T. M. J Appl Polym Sci 2003, 87, 1525.