Spectral Sensitivity of Chitosan Photodegradation

ANTHONY L. ANDRADY,[1] AYAKO TORIKAI,[2] and TAKAHIRO KOBATAKE[2]

[1]Camille Dreyfus Laboratory, Research Triangle Institute, Research Triangle Park, North Carolina 27709, and [2]Nagoya University, Nagoya, Japan

SYNOPSIS

The wavelength sensitivity of photodegradation of solvent-cast chitosan films exposed to monochromatic UV-visible radiation is reported. Measurements were made of changes in absorption spectra, both in the UV-visible region and in the infrared region, as well as changes in dilute solution viscosity of samples, on irradiation at selected wavelengths. Action spectra are reported for these processes. A mechanism of photodegradation based on changes in Fourier transform infrared (FTIR) spectra of irradiated chitosan is presented.

INTRODUCTION

Chitin is the second most abundant naturally occurring biopolymer (after cellulose) and is found in a variety of biosystems, including fungal cell walls, the exoskeleton of crustaceans, skeletal tissue of mollusks, and the integument of insects. Chitin is mainly poly(β-(1-4)-2-acetamido-D-glucose) and is structurally identical to cellulose, except that the secondary hydroxyl on the α carbon atom is substituted by an acetamide group.[1,2] Chitosan is derived from chitin by deacetylation. It is thus a generic product which is a copolymer consisting of (β-(1-4)-2-acetamido-D-glucose) units and β-(1-4)-2-amino-D-glucose) units, with the latter usually exceeding 80%. Chitosans are thus described in terms of the degree of deacetylation and the average molecular weight. They form clear, tough, water-insoluble films and have been proposed as a wet-strength additive in paper for packaging applications, nonwoven fabric, fibers, and other uses.[1] Some of these uses involve the routine exposure of the biopolymer to light with possible photodegradation of the polymer.

The photodegradation behavior of chitosans has not hitherto been reported. However, because of its structural similarity to cellulose and because it absorbs UV-A and UV-B ultraviolet radiation, some photodegradation at terrestrial solar wavelengths might be expected. Solar wavelengths are able to dissociate the glycosidic linkages in cellulose, causing a loss in strength of the polymer films due to reduced average degree of polymerization. Electron spin resonance (ESR) spectroscopic evidence suggests the process to be a free-radical mediated chain scission at the C-1 and C-4 positions in cellulose.[3] In addition to a parallel chain scission process, chitosan may also undergo photodegradation reactions involving the amine group. Cellulose photodegradation is well known to be wavelength sensitive; *in vacuo,* wavelengths longer than 340 nm have little effect on cellulose. In the presence of oxygen, however, photodegradation and free-radical formation is observed.[4] With UV-B, irradiation dehydrogenation, preferentially at the C-1 and C-5 positions, takes place.[3] The wavelength sensitivity is illustrated further by the effects of different light sources on the rate of photodegradation of cellulose.[5] Similarity in structure of the two biopolymers, cellulose and chitosan, suggest these mechanisms to be available to the latter as well. For the same reasons, it is reasonable to expect wavelength sensitivity in the photodegradation of chitosans as well.

The present study was undertaken to investigate the basic features of chitosan photodegradation, particularly the spectral sensitivity of degradation on exposure to monochromatic radiation.

Journal of Applied Polymer Science, Vol. 62, 1465–1471 (1996)
 CCC 0021-8995/96/091465-07

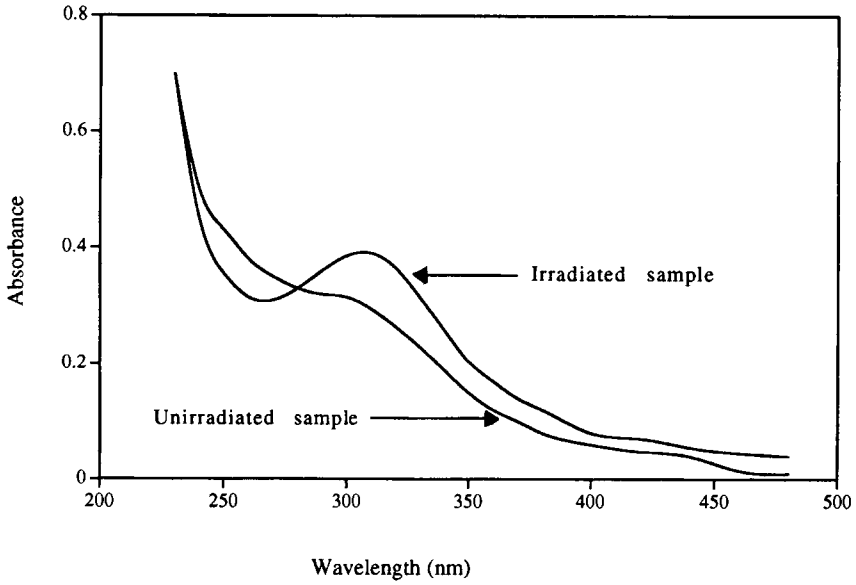


Figure 1 The UV-visible absorption spectrum for a chitosan film before and after irradiation at 260 nm.

EXPERIMENTAL

Chitosan Film Preparation

Commercial chitosan flakes from Protan Corporation were dissolved in 1% acetic acid solution with slow stirring overnight. The viscous solution was filtered by pressing through several layers of cloth to remove insoluble gel and other particles. The acid solution containing approximately 2.5 wt % chitosan was degassed for 1 h in a vacuum oven and cast as films on clean glass plates using a gardner knife at ambient temperature. The cast films were placed on a horizontal surface at a temperature of 60°C for several hours. Dried films peeled off easily with a razor blade and were soaked in 1*N* sodium hydroxide for several hours to neutralize excess acid. The neutralized films were washed thoroughly with cold water until the washings were neutral to litmus and air-dried at ambient temperature in the dark. The samples were further dried in an oven at 60°C for about 4 h and stored dry in a refrigerator until use.

Monochromatic Exposure Studies

The thickness of individual film samples were measured with a micrometer gauge and mounted in paperboard sample holders. An area of about 2.5 × 1.5 cm of the film was exposed in the holder.

The design and operation of the Okazaki Large Spectrograph (Okazaki, Japan) has been reported previously.[6] It is essentially a 30 kW xenon-arc source, whose radiation is diffracted through a 1200

Table I Average Changes in Absorbance at 310 nm/Photon and Solution Viscosity/Photon for Chitosan Films Exposed to Monochromatic Radiation

Wavelength (nm)	Mean Photons × 10 E-19	Mean Absorbance at 310 nm/Photon	Std. Error	Change in Solution Viscosity/Photon
260	3.31	7.88 10E-20	7.17 10E-21	5.78 10E-20
280	10.0	1.88 10E-20	3.48 10E-21	2.64 10E-20
300	15.6	−1.93 10E-21	3.35 10E-21	4.15 10E-21
320	18.0	−1.05 10E-21	2.10 10E-21	9.55 10E-21
340	17.6	−2.96 10E-21	6.27 10E-22	2.84 10E-22
360	7.78	3.15 10E-21	1.03 10E-21	1.29 10E-24
380	9.96	5.02 10E-21	1.34 10E-21	7.83 10E-22
400	17.0	2.41 10E-21	8.11 10E-22	2.48 10E-21
500	15.6	1.01 10E-21	3.96 10E-22	7.28 10E-21

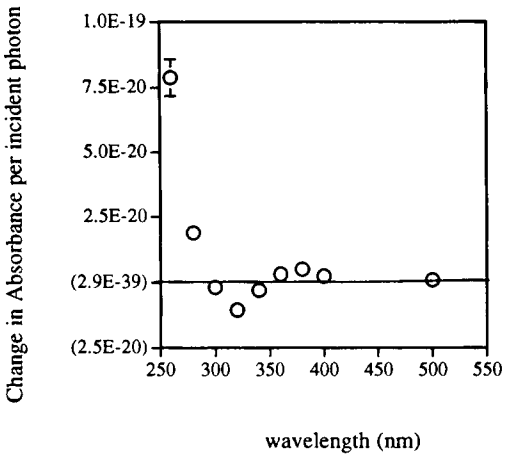


Figure 2 Change in absorbance of chitosan films at 310 nm per photon incident on sample surface as a function of the wavelength of irradiation.

lines/mm double-blazed grating to obtain a focused spectrum on a 10 m long focal curve. This allows resolutions of 1 nm per cm or less and affords an excellent monochromatic radiation source for exposure experiments. The samples of chitosan were placed at different points on the focal curve to obtain eight different wavelengths: 260, 280, 300, 320, 340, 360, 400, and 500 nm. Two monochromatic exposures were conducted; five samples per wavelength were exposed simultaneously in the first experiment, and only two samples per wavelength were used in the second exposure. The total photons per square cm of the sample was approximately 10E20 at each wavelength.

The exposure was discontinued at predetermined exposure times, and the samples were wrapped in black paper. Testing of the samples were carried out at Nagoya University and Research Triangle Institute.

Spectroscopic Studies

UV-visible spectroscopy of the exposed control samples was carried out using a Hitachi Model 323 spectrophotometer. The FTIR studies were made on a JASCO 5300 FTIR spectrophotometer using attenuated total reflection (ATR) method with the samples placed on a KSR-5 sample prism.

Viscosity Measurements

Viscosity determinations were made using an Ubbeholde viscometer on solutions of chitosan in 2% acetic acid in distilled water buffered with 1% sodium acetate. A sample of 15–30 mg of chitosan film exposed at a given wavelength was dissolved in 15 mL of solvent for viscosity determinations. The average flow times based on three determinations were converted into viscosity estimates.

RESULTS AND DISCUSSION

Chitosan solutions are known to absorb in the UV-visible region with a broad maximum at about 290–300 nm.[7] Cellulose also shows an absorption band, but at about 260 nm, which increases in intensity on exposure to UV radiation.[8] As seen from the absorbance spectrum in Figure 1, chitosan films absorb strongly in the UV. On exposure to monochromatic radiation (approximately 2 × 10E20 photons), the absorption spectrum was altered with a distinct absorption band at 310 nm being formed. The changes were more apparent at the shorter UV wavelengths. Table I lists the change in absorbance at 310 nm per mm per photon for exposed chitosan films, obtained at different irradiating wavelengths. Each data point was calculated from the average value of the change in absorbance of seven samples and the average of the respective photon fluences of the samples. As might be expected, the efficiency of photodamage decreases sharply with the wavelength of irradiation. For several wavelengths in the UV-A region, the absorbance of irradiated samples is lower than that of the control samples (see Fig. 2). The chromophores responsible for the absorption band are apparently destroyed by longer wavelength radiation but are generated at the lower wavelengths.

Spectral sensitivity data for synthetic polymers have often been represented graphically as plots of

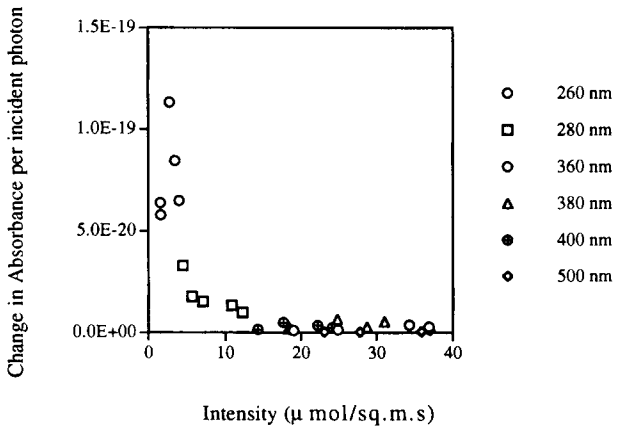


Figure 3 Intensity dependence of the efficiency of photodegradation as measured by the change in absorbance at 310 nm of chitosan, obtained at different irradiating wavelengths.

Table II Intensity Dependence of the Change in Absorbance of Chitosan Films on Irradiation with UV-B Radiation[a]

Wavelength (nm)	Intensity (μmol/cm^2 s)	Effectiveness $\times 10^{20}$	Wavelength (nm)	Intensity (μmol/cm^2 s)	Effectiveness $\times 10^{20}$
260	3.99	6.49	280	12.3	0.97
260	3.45	8.45	280	10.9	1.32
260	2.75	11.30	280	7.13	1.49
260	1.60	5.78	280	5.67	1.75
260	1.53	6.38	280	4.48	3.28

[a] Effectiveness = change in absorbance at 310 nm per incident photon.

the natural logarithm of damage per available photon versus the irradiation wavelengths.[9,10] Photon energy varies approximately exponentially in the experimental wavelength interval of 260 to 600 nm. In the case of photoprocesses whose effectiveness varies with photon energy, such a plot will therefore be linear. In spite of the complicated dependence of photodegradation on wavelength, seen in Figure 2, those samples which yielded an increase in absorbance at 310 nm on irradiation did show such a logarithmic dependence (gradient 0.017 and $r^2 = 0.89$).

The effectiveness of a photoreaction (photodamage per photon incident on the sample) may vary with the intensity of radiation, depending on whether the reciprocity rule is obeyed by the system. About seven samples of chitosan were exposed at each wavelength for about the same total photon fluence but at different intensities. The replicate data was studied for indications of intensity dependence of the change in absorbance at 310 nm. As shown in Figure 3, only 280 and 260 nm samples showed significant intensity dependence of photodegradation. Even in these cases, the span of intensity levels employed was too narrow to allow any firm conclusions to be drawn from the data. The data for exposure at 280 nm suggest the effectiveness to decrease with increased intensity. This is plausible in the case of a free radical photoprocess when higher radical concentrations increase the probability of termination and shorter kinetic chain lengths. Chitosan may undergo free-radical photodegradation via mechanisms similar to those shown by cellulose. The scatter in data and the limited range of photon fluence does not justify quantitative treatment of the data. Table II shows the data at the two UV-B wavelengths to illustrate the trend and the degree of scatter.

Based on ESR studies, photodegradation of cellulose is believed to involve chain scission, dehydroxylation or dehydroxymethylation, and scission of bonds in the ring.[11,12] Chain scission in chitosan might be expected via a process similar to that in cellulose, the photolysis of the glycosidic linkage.

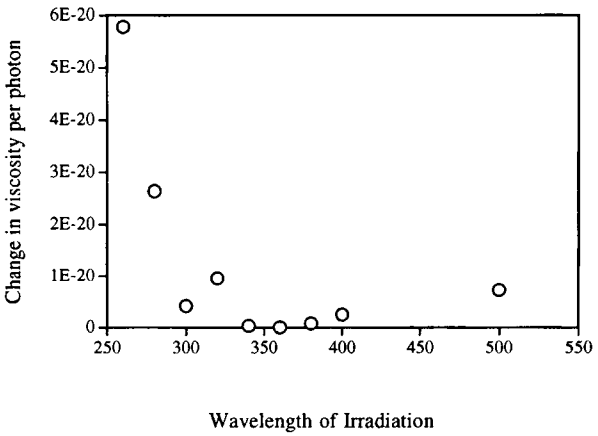


Figure 4 Change in dilute solution viscosity (in dilute acetic acid) of chitosan samples per incident photon as a function of the wavelength of irradiation of sample.

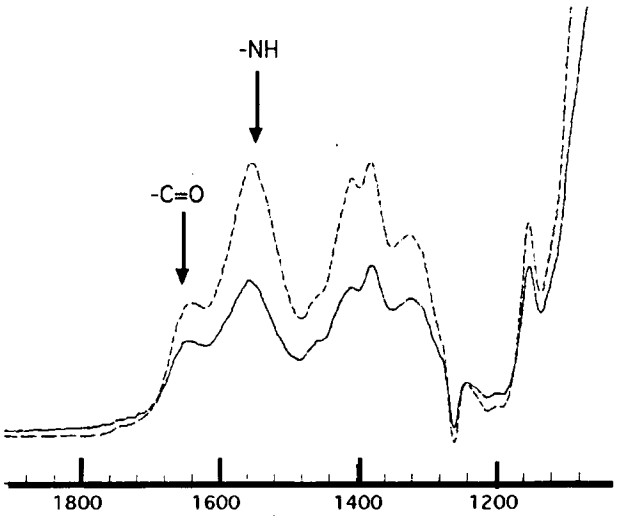


Figure 5 Sections of a typical FTIR spectrum of chitosan film samples before and after irradiation at 260 nm.

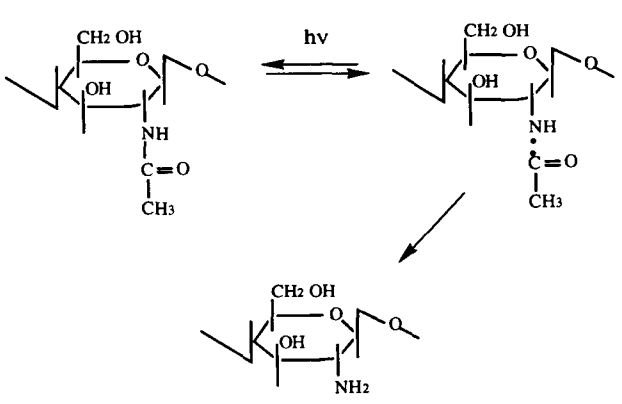
Table III Relative Intensity of Absorption Bands in the FT IR Spectrum of Irradiated and Unirradiated Chitosan Samples

λ (nm)	Ratio of Optical Density[a]			
	1640 cm^{-1}/2920 cm^{-1}		1550 cm^{-1}/2920 cm^{-1}	
	Unirradiated	Irradiated	Unirradiated	Irradiated
260	1.00	1.32	1.87	1.85
	1.13	1.33	1.75	1.71
280	0.95	1.25	1.71	1.57
	1.10	1.31	1.60	1.73
300	1.14	1.27	1.71	1.79
	1.05	1.24	1.52	1.68
320	0.95	1.23	1.40	1.58
	0.96	1.27	1.43	1.62
340	1.06	1.21	1.49	1.72
	0.96	1.21	1.47	1.60
360	1.00	1.20	1.39	1.72
	0.99	1.18	1.37	1.60
380	1.01	1.14	1.58	1.54
	1.05	1.17	1.47	1.60

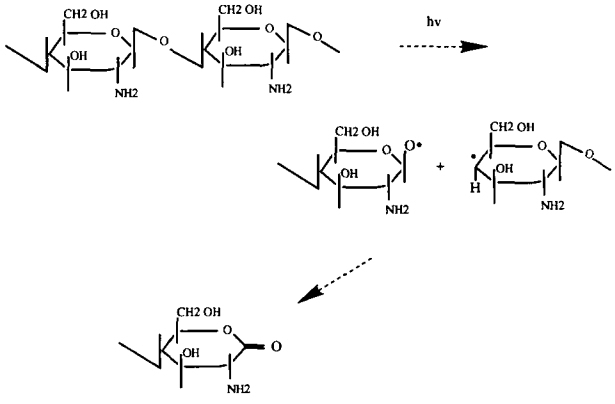
[a] Based on FT IR spectra for the same sample film before and after irradiation.

Even small amounts of main-chain scission of the polymer must result in a measurable drop in the solution viscosity of the material. Figure 4 shows a plot of the data given in Table I. As 15–30 mg/ml concentrations of irradiated chitosan samples were required for viscosity determination, several samples exposed at the same wavelength had to be combined for the purpose. Therefore, the plot shows single data points for viscosity plotted against the wavelength of irradiation. Wavelengths shorter than 340 nm yielded a measurable reduction in dilute solution viscosity, indicating chain scission during irradiation. Insoluble gel was not detected during dissolution, and cross-linking can therefore be ruled out as a significant process under present exposure conditions. Viscometric data is generally interpreted in terms of viscosity-average molecular weight changes using Mark Houwink relationship.[13] With a polyelectrolyte, such as chitosan, the validity of the viscometric relationship is questionable,[14] and the choice of the numerical values of Mark Houwink constants are not clear.[15] Data is therefore not converted to molecular weight estimates or scission effectiveness estimates.

The photochemical changes are also reflected in the FTIR spectra of the biopolymer. Figure 5 shows



Scheme I



Scheme II

a typical FTIR spectrum of a sample film of chitosan prior to exposure. The vibration of $-NH_2$ group is responsible for the absorption band at about 1560 cm^{-1}, and the carbonyl absorption is seen as a shoulder on the upfield side of the peak at about 1640 cm^{-1}. The changes in the spectrum obtained at each wavelength might be better appreciated by observing the change in the intensity of these peaks relative to a reference peak before and after irradiation. A convenient internal reference peak[16] is the —CH vibration attributed to the pyranose ring, which appears at about 2950 cm^{-1}. This band is tentatively assigned to the CH stretching vibration of the pyranose ring. The peak heights were calculated from FTIR data for irradiation wavelengths between 260 and 380 nm for samples from two experiments. The data is given in Table III.

In a study of spectral changes induced in chitosan exposed to γ radiation, the amide band at 1653 cm^{-1} was used as the reference peak.[17] However, with present samples, the 2920 cm^{-1} band is preferred as the amide functionalities might be expected to undergo photodegradation.

As the spectra of samples prior to irradiation are essentially replicates, the ratios were averaged to obtain mean values of 1.01 (std. error 0.019) for the 1640/2920 band and 1.55 for the 1550 cm^{-1}/2920 cm^{-1} band. The data in Table III show a significant increase in the intensity of the 1640 cm^{-1} band attributed to carbonyl absorption for all wavelengths of irradiation but becomes less pronounced with longer wavelength of irradiation (see Fig. 5). While relatively less pronounced, the 1550 cm^{-1} peak attributed to amino groups also increases in intensity significantly at wavelengths longer than about 320 nm. These conclusions assume the C—H bonds in pyranose ring to be unaffected by photodegradation.

In spite of the lability of C—N bond affixing the amine group to the ring (69.7 kcal/mol), there appears to be no loss of amine functionality on irradiation of the polymer. The increase in amine might be due to photodegradation of the pyranose ring, decreasing the reference peak, or due to possible conversion of (β-(1-4)-2-acetamido-D-glucose) units to glucosamine units. The C—N bond in the amide group is weak (about 53 kcal/mol) and can be photolyzed, and the resulting radical may abstract hydrogen to form a primary amine. While this mechanism is observed in the case of both aliphatic and aromatic polyamides at 254 nm,[17] it is less likely to occur at longer wavelengths.

However, particularly at longer ultraviolet wavelengths, pathways which yield carbonyl functionalities via macrooxy radicals from scission reaction are possible. Analogous reactions have been postulated for cellulose.

The observed reduction in viscosity of solutions of irradiated chitosan is consistent with the occurrence of main chain scission. Qualitatively, irradiation is seen to increase the relative intensity of the carbonyl absorption band at all irradiation wavelengths. Ring cleavage, which can also yield an increase in carbonyl content, has been postulated in the case of UV irradiation of cellulose.[18]

CONCLUSIONS

Exposure of solid chitosan film samples to monochromatic radiation results in an increased absorbance (measured at 310 nm) in the UV-visible spectrum and a reduction in the dilute solution viscosity of the polymer. Both changes were shown to be wavelength-dependent, with the most drastic photodegradation occurring at wavelengths shorter than 360 nm. The viscosity changes are interpreted in terms of photolytic main-chain scission of the polymer, while changes in absorbance is a result of the accumulation of chromophores. The spectroscopic evidence suggests an increase in the carbonyl and amino groups in the polymer after irradiation. While plausible mechanisms to explain these changes exist, further work is needed to elucidate a complete mechanism.

We thankfully acknowledge the support and guidance of Professor Masakatsu Watanabe and Mr. Mamoru Kubota (NIBB) in exposure experiments and Ms. Song Ye (RTI) for preparing the samples of chitosan. This study was carried out under the NIBB Cooperative Research Program for the Okazaki Large Spectrograph (95-527).

REFERENCES

1. R. A. A. Muzzarelli, C. Jeuniaux, and G. W. Gooday, *Chitin in Nature and Technology,* Plenum Press, New York, 1986.
2. R. A. A. Muzzarelli, *Chitin,* Pergamon Press, Oxford, 1977.
3. N.-S. Hon, *J. Macromol. Sci., Chem.,* **A10,** 1175 (1976).
4. T. N. Kleinhart, *Holzforsch,* **18,** 24 (1964).
5. N.-S. Hon, *J. Polym. Sci., Polym. Chem. Ed.,* **13,** 1347 (1975).
6. M. Watanabe, M. Furuyu, Y. Miyoshi, I. Iwahashi, and K. Matsumoto, *Bioscience,* **33,** 53 (1983).

7. P. Broussignac, *Chim. Ind. Gen. Chim.*, **99,** 1241 (1968).
8. N.-S. Hon, *Photodegradation of Cellulose,* Applied Science, New Jersey, 1981.
9. A. L. Andrady, A. Torikai, and K. Fueki, *J. Appl. Polym. Sci.*, **37,** 935 (1989).
10. A. L. Andrady, N. D. Searle, and L. F. E. Crewdson, *Polym. Deg. Stab.*, **35,** 235 (1992).
11. Y. Merle and J. P. Fouassier, *Makromol. Chem.*, **108,** 185 (1982).
12. Y. Merle and J. P. Fouassier, *Angew Makromol. Chem.*, **86,** 109 (1980).
13. P. J. Flory, *Principals of Polymer Chemistry,* Cornell University Press, Ithaca, 1953.
14. A. Domard and M. Rinaudo, *Int. J. Biol. Macromol.*, **5,** 49 (1983).
15. G. A. F. Roberts and I. Domzty, *Int. J. Biol. Macromol.*, **4,** 374 (1982).
16. A. Takahashi, Y. Sugaharo, and Y. Horikawa, *Sen-I Gakkashi,* **43,** 76 (1987).
17. Z. Wenwei, Z. Xiaoguang, Y. Li, Z. Yuefang, and S. Jiazhen, *Polym. Deg. Stab.*, **41,** 83 (1993).
18. J. F. Rabek, in *Polymer Photodegradation. Mechanisms and Experimental Methods,* Chapman and Hall, New York, 1995, p. 344.

Received December 18, 1995
Accepted May 30, 1996