Role of Green Tea Polyphenol Crosslinking in Alleviating Ultraviolet-Radiation Effects on Collagen

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ABSTRACT: The widespread application of collagen warrants studies on the effects of ultraviolet (UV) radiation on stabilized collagen. The negative impact of UV radiation is well known. Because collagen is used as a biomaterial in various biomedical applications, knowing the effects of UV irradiation on stabilized collagen has become essential. In this study, the effects of UV irradiation on collagen stabilized with green tea polyphenols, that is, *Acacia mearnsii* (wattle), and catechin has been studied. The fluorescence intensity has been found to decrease with irradiation for native and wattle-treated collagen. Spectral studies have indicated that the photodegradation products increase after irradiation for native collagen, whereas collagen treated with catechin or *A. mearnsii* exhibits different responses depending on the duration of the irradiation. The duration of the irradiation has a significant influence on polyphenol-treated collagen. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 3382–3386, 2007

Key words: antioxidants; fluorescence; irradiation; proteins; UV-vis spectroscopy

INTRODUCTION

Collagen is the main protein of connective tissue in animals and the most abundant protein in mammals, making up about 40% of the total. It is a long, fibrous structural protein with great tensile strength and is the main component of cartilage, ligaments, tendons, bones, and teeth. Along with soft keratin, it is responsible for skin strength and elasticity, and its degradation leads to the wrinkles that accompany aging.[1]

Ultraviolet (UV) rays are part of the electromagnetic spectrum that can reach a high enough level on earth to be harmful to plants, animals, and humans. Wavelengths in the UVB region (280–320 nm) of the solar spectrum are absorbed by the skin, producing erythema, burns, and eventually skin cancer. UVA (320–400 nm) is supposed to be weakly carcinogenic and cause aging and wrinkling of the skin.[2] Despite its harmful effects, it has been reported that the process of UV irradiation can induce crosslinks in collagen fibrils. Molecular scission through free-radical mechanisms has also been reported. UV radiation has been shown to induce both chemical and physical changes in collagen. The thermal helix–coil transition of UV-irradiated collagen in rat tail tendons has been investigated with differential scanning calorimetry. The aromatic groups of collagen, phenylalanine and tyrosine, have been found to be affected by UV irradiation.[3–5] Fujimori[6] showed that collagen undergoes photopolymerization under irradiation and that this takes place in the telopeptide regions of the molecule.

Polyphenols are a group of chemical substances found in plants and characterized by the presence of more than one phenol group per molecule. *Acacia mearnsii* (wattle) is a type of condensed tanning agent that is used widely in tanning industries. *A. mearnsii* is also used in making adhesives, precipitants for clay suspensions, mud-thinning agents for oil-well drilling, and surface coatings for woods.[7] *A. mearnsii* contains a soup of polyphenols. Catechin is the main constituent of *A. mearnsii*. Research suggests that polyphenols are antioxidants with potential health benefits. Polyphenols may reduce the risk of cardiovascular disease and cancer. Sources of polyphenols include green tea, white tea, olive oil, dark chocolate, pomegranates, and other fruits and vegetables.[8–10] Researchers believe that catechin has the capacity to quench harmful UV radiation.[11] Catechin is also known as an anticancer chemopreventive agent and has been used for medical purposes in the form of tea drinking. The high antioxidant activity of green tea makes it beneficial for protecting the body from oxidative damage due to free radicals. Research has shown that green tea may help the arterial wall by reducing oxidized lipids.[12] Catechin is effective because it easily sticks to proteins, blocking bacteria from adhering to cell walls and disrupting their ability to destroy them. Viruses have hooks on their

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surfaces and can attach to cell walls. The catechin in green tea prevents viruses from adhering and causing harm. Catechin reacts with toxins created by harmful bacteria (many of which belong to the protein family) and harmful metals such as lead, mercury, chrome, and cadmium.[13,14]

This article reports experiments investigating the effects of UV irradiation on the physicochemical properties of collagen treated with condensed polyphenol *A. mearnsii* (wattle) and its main constituent catechin because of their applications and significance in both industrial and medical fields.

EXPERIMENTAL

Collagen solutions

Collagen solutions were prepared from tendons freshly dissected from the tails of 6-month-old male albino rats frozen at −20°C by acetic acid extraction and salting-out with NaCl.[15] The purity of the collagen preparation was confirmed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis; the bands appearing in the gel corresponded only to type 1 collagen. The collagen concentration in the solutions was determined from the hydroxyproline content.[16] The average molecular weight of collagen was 300,000 Da, on the basis of which the molar concentration was determined. The stock concentration of the prepared collagen was 3 μ*M*. The polyphenol treatment was carried out through the use of the required molar concentration of polyphenols with a collagen solution for 24 h at pH 4.0 and 25°C without any mechanical agitation. The concentration of the polyphenols was based on a collagen/polyphenol molar ratio of 1 : 100.

UV irradiation

The solutions were irradiated under air at room temperature with a quantum yield photoreactor (model 2001, Applied Photophysics, Ltd., London, England) with a 250-W medium-pressure mercury lamp, which emitted light mainly at a wavelength of 330 nm. Irradiation experiments were carried out in a quartz cuvette at a distance of 20 cm from the light source for various times. All measurements were performed under the same temperature and humidity conditions to avoid any influence on the physicochemical properties of collagen.

UV–vis spectral studies

The UV absorption spectra for native and polyphenol (wattle and catechin)-treated collagen solutions before and after irradiation were recorded with a PerkinElmer (Waltham, MA) Lambda 35 spectrophotometer. The concentration of collagen was 0.6 μ*M*. The molar ratio of collagen to polyphenols was maintained at 1:100.

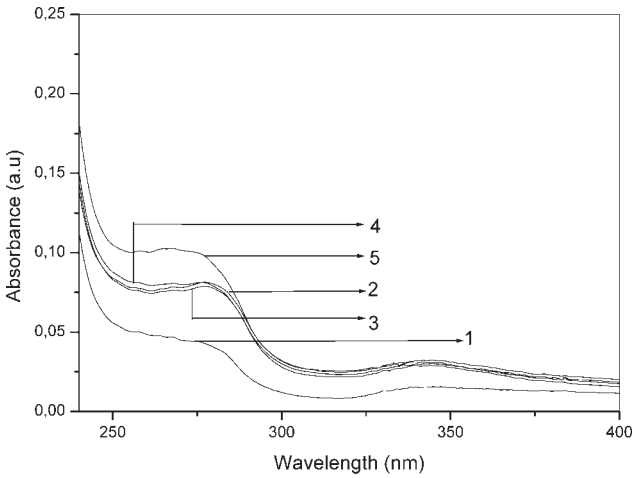

Figure 1 UV absorption spectra for a native collagen solution before and after irradiation: (1) 0, (2) 15, (3) 30, (4) 60, and (5) 120 min.

Fluorescence studies

The emission spectra for native and polyphenol-treated collagen solutions before and after irradiation were recorded with a Cary Eclipse fluorescence spectrophotometer from Varian (CA, USA). The solutions were excited with light of a wavelength of 270 nm, and the emission at 290 nm was monitored. The concentration of collagen was 0.6 μ*M*. The molar ratio of collagen to polyphenols was maintained at 1 : 100.

RESULTS AND DISCUSSION

Collagen possesses unique characteristics as a biomaterial that are distinct from those of other macromolecules and hence is a widely used biomaterial in various biomedical applications. Radiation is known to bring about both crosslinking and molecular scission. In this study, the role of polyphenol crosslinking in imparting stability to collagen against UV irradiation has been studied with various physicochemical techniques. Polyphenols act as antioxidants, and their interaction with collagen has been found to impart stability to collagen.[17]

The electronic absorption spectra for collagen before and after irradiation are presented in Figure 1. Electronic absorption spectra for untreated catechin and catechin-crosslinked collagen before and after irradiation are presented in Figure 2(a,b), respectively. The same for wattle is shown in Figure 3(a,b). There is a peak centered around 275 nm that is characteristic of tyrosine (Fig. 1). The intensity of the peak increases with increasing irradiation time. This

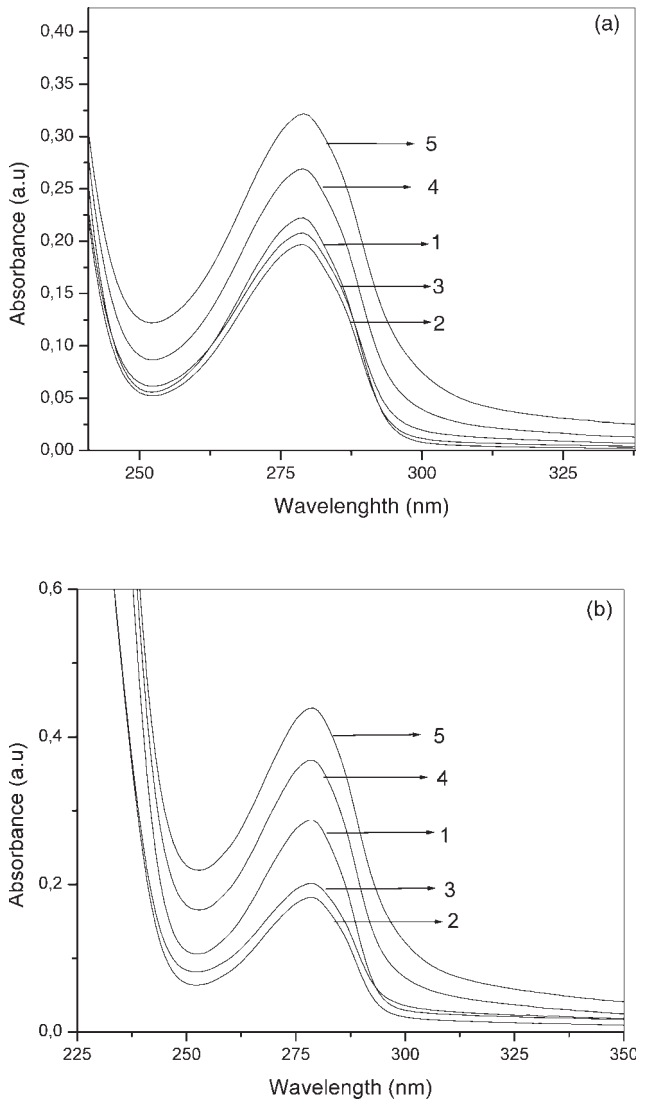

Figure 2 UV absorption spectra for (a) an untreated catechin solution and (b) a collagen solution in the presence of catechin before and after irradiation: (1) 0, (2) 15, (3) 30, (4) 60, and (5) 120 min.

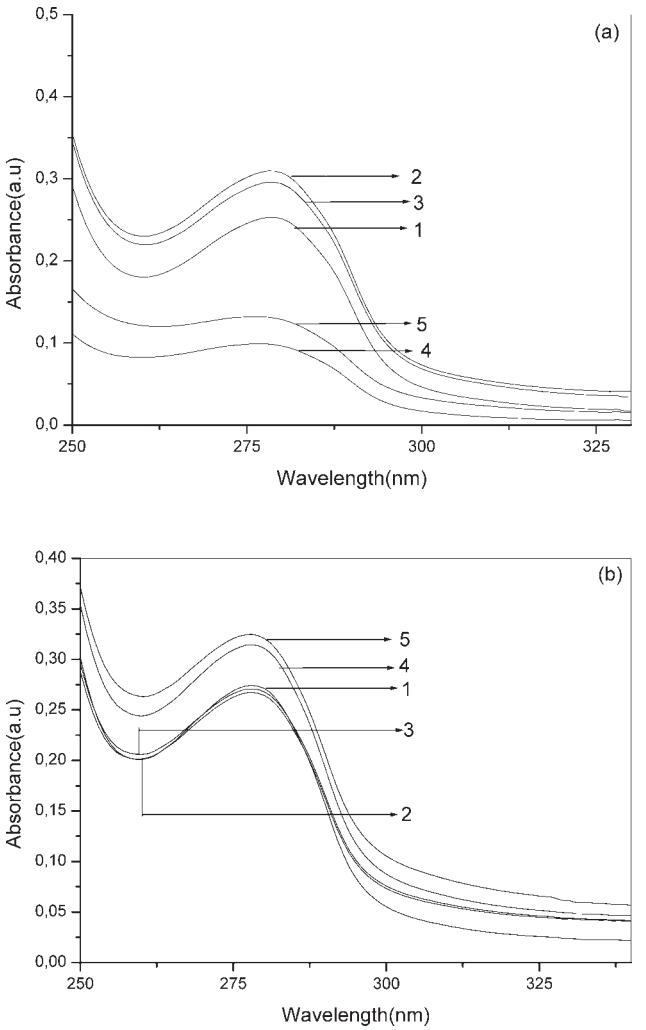

Figure 3 UV absorption spectra for (a) a wattle solution and (b) a collagen solution in the presence of wattle before and after irradiation: (1) 0, (2) 15, (3) 30, (4) 60, and (5) 120 min.

can be attributed to the increase in photoproducts formed by irradiation of the aromatic amino acids tyrosine and phenylalanine. Swallow[18] observed that in proteins containing more tyrosine units than tryptophan, the optical absorbance of an irradiated solution around 280 nm shows an increase like that observed from the irradiation of tyrosine. This shows that more UV-absorbing centers are formed after irradiation.

However, the effect of UV irradiation on catechin-treated collagen is different. As shown in Figure 2, the absorbance decreases after 15 min of irradiation, and this is followed by an increase after 30 min of irradiation. This increase is, however, less than the absorbance for nonirradiated catechin-treated collagen. A prolonged time of irradiation has been found to further increase the absorbance. Figure 3(a) shows

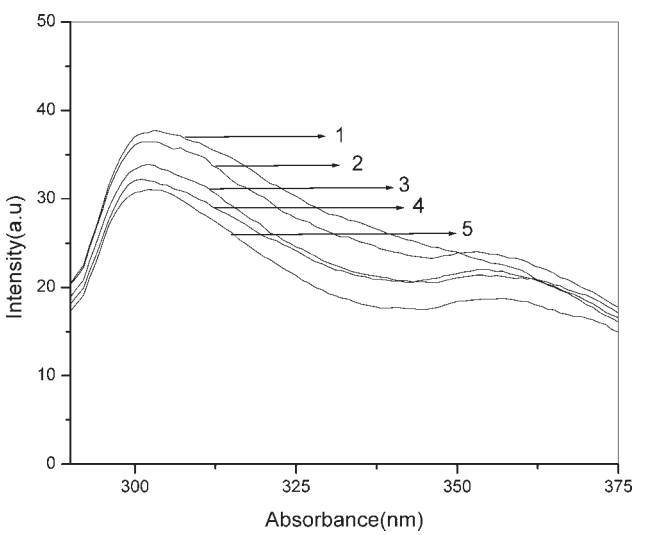

Figure 4 Effect of UV irradiation on the fluorescence spectra of a native collagen solution before and after irradiation: (1) 0, (2) 15, (3) 30, (4) 60, and (5) 120 min.

the effect of UV irradiation on untreated wattle. The initial time of irradiation has resulted in an increase in the absorbance followed by a decrease after 1 and 2 h of irradiation. The reaction of wattle-treated collagen [Fig. 3(b)] to UV radiation is different from that of wattle alone [Fig. 3(a)]. That is, 15 or 30 min of irradiation has been found to increase the absorbance only marginally; 1 or 2 h of irradiation has been found to increase the absorbance significantly, unlike the decrease in the absorbance for the wattle alone. This difference could be due to fact that wattle, which contains various constituents of polyphenols, interacts with collagen and hence responds to UV light differently.

The fluorescence emission spectra of collagen before and after irradiation are shown in Figure 4. The fluorescence emission spectra of untreated polyphenols and polyphenol-crosslinked collagen, before and after irradiation, are shown in Figures 5(a,b) and 6(a,b), respectively. With increasing irradiation time, there is a gradual decrease in the emission maxima at 300 nm when the excitation wavelength is 270 nm for native collagen. The catechin-treated collagen also responds to the UV irradiation in the same way as native collagen [Fig. 5(b)]. The same is observed in the case of wattle-treated collagen [Fig. 6(b)]. A loss of tyrosine residue and the formation of dityrosine molecules have been reported in collagen after UV irradiation.[19]

Polyphenols interact with collagen primarily through hydrogen bonding. A collagen molecule contains various functional groups such as side-chain hydroxyl groups of the amino acids serine and hydroxyproline, carboxyl groups of aspartic acid,

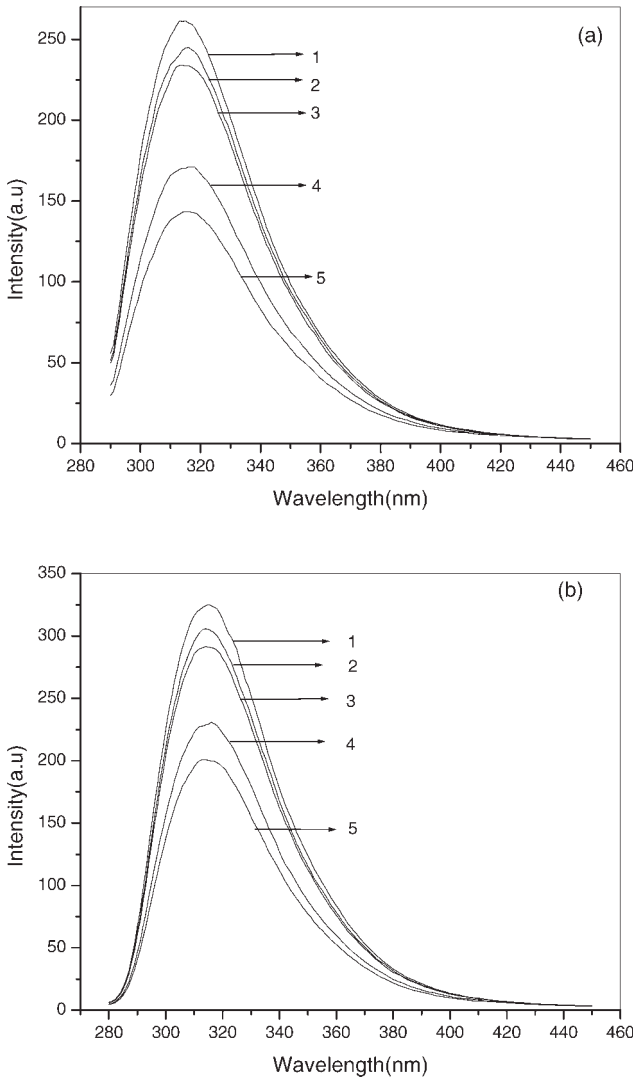

Figure 5 Effect of UV irradiation on the fluorescence spectra of (a) a catechin solution and (b) a collagen solution in the presence of catechin before and after irradiation: (1) 0, (2) 15, (3) 30, (4) 60, and (5) 120 min.

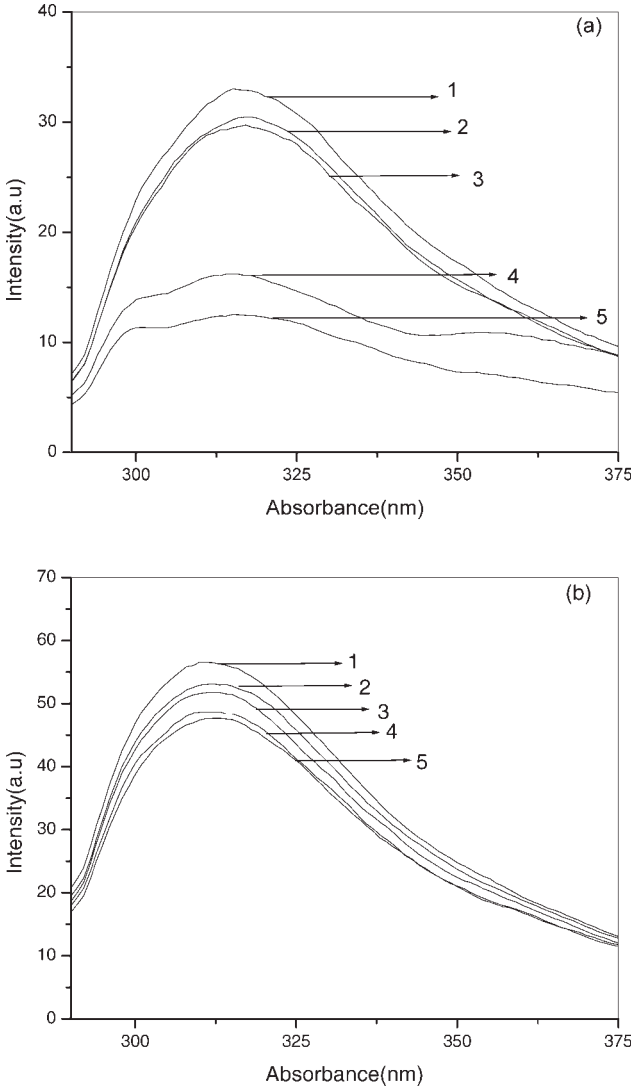

Figure 6 Effect of UV irradiation on the fluorescence spectra of (a) a wattle solution and (b) a collagen solution in the presence of wattle before and after irradiation: (1) 0, (2) 15, (3) 30, (4) 60, and (5) 120 min.

amino groups of lysine, and amide groups of asparagine, which are considered potential interacting sites for the formation of hydrogen bonds with the polyphenols. The mechanism of the interaction of catechin with collagen has been elucidated.[17] Catechin-treated collagen fibers are stable even after a treatment with urea, a known protein denaturant. The role of green tea polyphenols in inhibiting the collagenolytic activity of collagenase has been reported recently.[20]

Polyphenols themselves are susceptible to UV radiation because they are organic molecules. Hence, blank experiments without collagen were carried out on wattle and catechin. There was higher absorbance of collagen-treated polyphenol than untreated polyphenol because of the presence of collagen. The trend remained the same with the irradiation of polyphenol in the absence of collagen. However, it has been found that the presence of polyphenols reduces the impact of UV irradiation on collagen.

CONCLUSIONS

This study throws light on the effect of UV irradiation on polyphenol-crosslinked collagen with respect to absorption and emission properties. The response of collagen to UV irradiation has been found to be different after a treatment with green tea polyphenols. This study has wider implications as polyphenols are present in food and are known to act as crosslinking agents for collagen.

References

1. Toyoshima, M.; Hosoda, K.; Hanamura, M.; Okamoto, K.; Kobayashi, H.; Negishi, T. J Photochem Photobiol 2004, 73, 59.
2. Chaqour, B.; Seite, S.; Coutant, K.; Fourtanier, A.; Borel, J. P.; Bellon, G. J Photochem Photobiol 1995, 28, 125.
3. Sionkowska, A. J Photochem Photobiol 2006, 82, 9.
4. Sionkowska, A.; Wess, T. Int J Biol Macromol 2004, 84, 9.
5. Sionkowska, A.; Kaczmarek, H.; Wisniewski, M.; Kowalonek, J.; Skopinska, J. Surf Sci 2004, 566, 608.
6. Fujimori, E. FEBS Lett 1988, 235, 98.
7. Roux, D. G. Phytochemistry 1972, 11, 219.
8. Higdon, J. V.; Frei, B. Crit Rev Food Sci Nutr 2003, 43, 89.
9. Henning, S. M.; Niu, Y.; Liu, Y.; Lee, N. H.; Hara, Y.; Thames, G. D.; Minutti, R. R.; Carpenter, C. L.; Wang, H.; Heber, D. J Nutr Biochem 2005, 16, 610.
10. Moncheva, S.; Trakhtenberg, S.; Katrich, E.; Zemser, M.; Goshev, I.; Toledo, F.; Arancibia-Avila, P.; Doncheva, V.; Gorinstein, S. Coastal Shelf Sci 2004, 59, 475.
11. Vaquero, M. J. R.; Alberto, M. R.; Manca, M. C.; Nadra, M. D. Food Control 2007, 18, 93.
12. Serafini, M.; Laranjinha, J. A.; Almeida, L. M.; Maiani, G. J Nutr Biochem 2000, 11, 585.
13. Wu, W. B.; Chiang, H. S.; Fang, J. L.; Chen, S. K.; Huang, C. C.; Hung, C. F. Life Sci 2006, 79, 801.
14. Tichopad, A.; Polster, J.; Pecen, L.; Pfaffl, M. W. J Ethnopharmacol 2005, 99, 221.
15. Chandrakasan, G.; Torchia, D. A.; Piez, K. A. J Biol Chem 1976, 251, 6062.
16. Woessner, J. F. J. Arch Biochem Biophys 1961, 93, 440.
17. Madhan, B.; Subramanian, V.; Rao, J. R.; Nair, B. U.; Ramasami, T. Int J Biol Macromol 2005, 37, 47.
18. Swallow, A. J. Radiation Chemistry; Longman: White Plains, NY; 1973; p 243.
19. Kato, Y.; Uchida, K.; Kawakishi, S. J Photochem Photobiol 1994, 59, 343.
20. Madhan, B.; Krishnamoorthy, G.; Rao, J. R.; Nair, B. U. Int J Biol Macromol 2007, 41, 16.