Effect of Electron Beam Irradiation on the Silk Fibroin Fiber/Poly(ε-caprolactone) Composite

Wei Li,[1] Xiuying Qiao,[1] Kang Sun,[1] Xiaodong Chen[2]

[1] *State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China*
[2] *Shanghai Sunny New Technology Development Co., Ltd., Shanghai 201108, China*

Received 9 May 2008; accepted 6 December 2008
DOI 10.1002/app.29869

ABSTRACT: The effect of electron beam irradiation on silk fibroin (SF) fiber-reinforced poly(ε-caprolactone) (PCL) composite was investigated by mechanical test, scanning electron microscope, dynamic mechanical thermal analysis, electron paramagnetic resonance (EPR) analysis, and Fourier transform infrared (FTIR) analysis. The results indicate that electron beam irradiation can affect the static and dynamic mechanical properties of PCL and SF/PCL composite, depending on the irradiation dose. PCL shows maximum strength and modulus at the irradiation dose of 200 kGy, and the SF/PCL composite with 45% fiber content exhibits maximum strength and modulus at the doses of 150 kGy. EPR analysis shows that during irradiation, both PCL and SF fiber can produce free radicals. Some transformations or reactions of radicals may take place between PCL and SF fiber and enhance the interfacial interaction. The microstructures of tensile fracture also show an improvement in interfacial interaction between fiber and matrix after irradiation. FTIR analysis shows that chain scissions also occur in both PCL and SF fiber during irradiation. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 1063–1069, 2009

Key words: poly(ε-caprolactone); (PCL); silk fibroin fiber; electron beam irradiation; free radicals

INTRODUCTION

Biodegradable polymer composites consisting of both biodegradable polymers and biodegradable fillers have got more and more attentions in recent years owing to their potential applications in biomedical and environmental fields. Among these composites, biocomposite based on poly(ε-caprolactone) (PCL) and reinforced by natural fibers is one of the leading examples.[1–6] As a kind of natural fiber, silk fiber (*Bombyx mori*) spun out from silkworm cocoons has excellent mechanical properties such as high tensile strength and modulus, high elongation, good elasticity, and excellent resilience.[7] *B. mori* silk fiber consists primarily of two protein-based components, the inner fibroin filaments and the outer sericin, which account about 75 and 25% by weight respectively.[8] Fibroin, a real fibrous component, is highly crystalline and well aligned in the fiber. Degumming is usually performed to achieve pure fibroin filaments with the removal of sericin component.[9,10] Biocomposites fabricated with PCL and silk fibroin (SF) fiber will have more promising foreground in biomedical fields, especially in bone substitute applications.

As a kind of biomaterials with potential use in biomedical fields, the SF/PCL composites must be sterilized before being used as biomedical applications. Irradiation treatment is an effective way for sterilization. Moreover, irradiation also can affect certain properties of polymers.[11] The influences of irradiation on the mechanical and optical properties, processability, and thermal stability of PCL were investigated by Yoshii and coworkers[12–14] Ohrlander et al.[15] and Filipczak et al.[16] discussed the formation of free radicals in PCL after irradiation through electron paramagnetic resonance (EPR) analysis. The effects of irradiation on PCL porous scaffolds were also studied as well as the bulk polymer.[17,18] Irradiation is also used as a tool for silk fiber modification and grafting.[19–22] Mamedov et al.[23] and Wang et al.[24] observed that EPR studies were also used to investigate the formation of free radicals in silk fiber after irradiation.

In our previous study, SF fiber-reinforced PCL biocomposites were fabricated, and the effect of fiber reinforcement was investigated by static mechanical test, dynamic mechanical thermal analysis (DMTA), and dynamic rheological analysis.[25] The results showed that SF/PCL composites with fiber content from 35 to 45% by weight had optimum mechanical behaviors. In this investigation, we choose SF/PCL composite with 45 wt % fiber content as an example to study the effect of electron beam irradiation on

---

Correspondence to: K. Sun (ksun@sjtu.edu.cn).

Journal of Applied Polymer Science, Vol. 113, 1063–1069 (2009)

the composites. After being irradiated by different dose, the mechanical properties, microstructures, and dynamic mechanical properties of SF/PCL composite were investigated by mechanical test, scanning electron microscope (SEM), and DMTA. The formations of free radicals and chain scissions in the SF/PCL composites irradiated with 150 kGy were discussed through EPR analysis and Fourier transform infrared (FTIR) analysis. The effect of irradiation on PCL was also studied to make a comparison.

EXPERIMENTAL

Materials and preparation

PCL with the number-average molecular weight of 90,000 was purchased from Solvay Group (Brussels, Belgium). Continuous silk fiber, spun by the cocoons of *B. mori*, was supplied by JinLi Silk Co. (YuHang, China). Before use, the silk fiber was chopped into short fiber with an approximate length of 1.5 cm in the lab.

To remove the sericins, the chopped silk was first degummed by boiling in the 0.5 wt % Na_2CO_3 water solution for 40 min, and then rinsing in deionized water and drying in vacuum at 70°C for 2 days.

SF/PCL composite with 45% fiber content by weight was prepared by melt-mixing PCL and SF fiber at 140°C in a Haake Rheocord900 Rheometer (Haake Mess-Technic GmbH, Germany) for 15 min, and then the obtained composite was hot pressed in a mold at 140°C for 10 min. PCL was also hot pressed in the same condition. Both PCL and composite were cut into suitable specimens for measurements.

The specimens were irradiated with a GJ-2 electron beam accelerator (Shanghai Xianfeng Corp., China) with a dose rate of 25 kGy/min at a room temperature in the presence of air. With the irradiation doses of 0, 25, 100, 150, 200, 250, and 300 kGy, the PCL and SF/PCL samples were denoted as PCL, PCL25, PCL100, PCL150, PCL200, PCL250, PCL300 and 45SP, 45SP25, 45SP100, 45SP150, 45SP200, 45SP250, 45SP300, correspondingly.

Measurement

An Instron2366 Universal Tensile Tester (Instron Corp., USA) was utilized for tensile and flexural tests. Both the loading rates of tensile and flexural measurements were chosen as 1 mm/min. The values of specimen dimensions for tensile and flexural measurements were 50 mm × 4 mm × 2 mm and 40 mm × 15 mm × 2 mm separately. The average values of tensile strength, elongation at break, flexural strength, and flexural modulus were determined from five test specimens to evaluate the tensile and flexural properties.

An S-2150 scanning electron microscope (Hitachi, Japan) was used to examine the tensile fracture morphology of untreated and irradiated SF/PCL composite. The cross sections were coated with a layer of gold before SEM observation.

DMTA was performed on a DMTA IV (Rheometric Scientific, USA) with a three-point bending mode at a fixed frequency of 1 Hz. The testing temperature was controlled from −100 to 30°C at a heating rate of 3°C/min. For each test, the dynamic mechanical property parameters of the storage modulus E' and the loss modulus E'' were obtained as a function of temperature under the condition of linear viscoelastic response.

EPR spectra of 150 kGy-irradiated PCL, silk fiber, and SF/PCL composite were obtained by the use of an EMX-8 EPR spectrometer (Bruker Biospin GmbH, Germany) with the microwave power of 0.2 mW. Measurements were performed at room temperature. First, the EPR signals of samples were recorded immediately after irradiation, and then followed by a second measurement after 1-month storage under ambient condition.

FTIR analysis of untreated and irradiated PCL and silk fiber was performed on an EQUINOX 55 FTIR spectrometer (Bruker Co., Germany) in an attenuated total reflectance mode.

RESULTS AND DISCUSSION

Mechanical properties

Figures 1 and 2 show the mechanical properties of PCL and SF/PCL composite with 45 wt % fiber content after being irradiated with different doses. From the figures, moderate irradiation dose can improve the tensile strength and flexural strength of both PCL and SF/PCL composite but too much dose can cause the decrease of strength. The flexural modulus of PCL and SF/PCL composite exhibits peak values at irradiation doses of 200 and 150 kGy, respectively. When irradiation dose is no more than 200 kGy, the elongation at break of PCL is above 200%, but when the irradiation dose is 250 and 300 kGy, the elongation at break of PCL decreases to 116.1 and 42.1%, respectively. As for the SF/PCL composite, the elongation at break also decreases remarkably after being irradiated.

The tensile properties of γ-irradiated PCL under the supercooled state were studied by Yoshii and coworkers[12] In their study, the tensile strength of PCL irradiated in the supercooled state got a maximum at 160 kGy. They attributed the decreasing of tensile strength at higher dose than 160 kGy to the more difficult rearrangement of polymer chains with high crosslink density during the extension process.

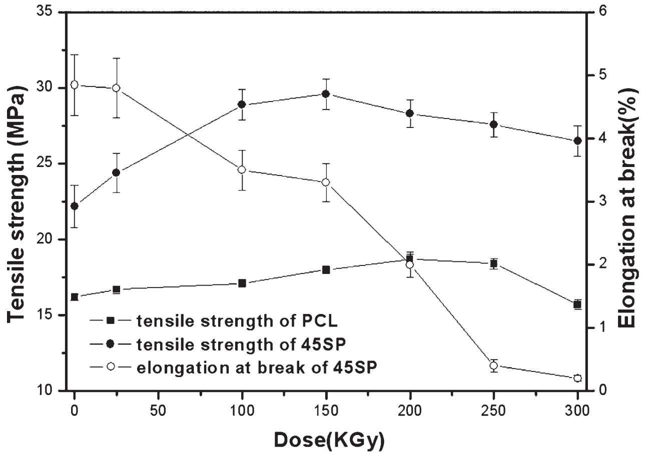

Figure 1 Effect of irradiation dose on the tensile properties of PCL and SF/PCL composites with 45% fiber content.

The crosslink of PCL also leads to a decrease in elongation at break. During irradiation, the crosslinking and chain scission in PCL occur side-by-side. At lower irradiation dose, the formation of crosslinking is predominant over chain scission and results in an enhancement of mechanical properties. While at higher irradiation dose, the mechanical properties decrease. Besides high crosslink density, chain scission is another reason. The extent of chain scission increases at higher dose and causes the degradation of PCL. In the SF/PCL composite, the rearrangement of polymer chains is hampered by SF fiber during the test and becomes more difficult than in pure PCL. This may result in that the peak values of flexural modulus of SF/PCL composite appear at 150 kGy, less than that of pure PCL.

Figure 3 shows the SEM photographs of the pulled-out fibers on the tensile fractures of 45SP, 45SP25, 45SP100, and 45SP150. For 45SP, the surface of fiber is smooth, whereas after being irradiated, the surface of fiber is irregular. The fiber is covered by a layer of PCL matrix and the extent of covering is more obvious as the irradiation dose increasing. The different morphology may be caused by the irradiation process, as discussed by Yang and Wu[26] about the irradiation effect on chitin fiber-reinforced PCL composite. The irradiation treatment may improve the interfacial bonding between SF fiber and PCL matrix. The strengthened bonding causes better load transition from PCL to SF fiber. The reinforcement effect of fiber becomes more effective. This may be another reason for the improvement of the mechanical properties of irradiated SF/PCL composites.

Dynamic mechanical thermal properties

Figures 4 and 5 show the variation of storage modulus (E') and loss modulus (E'') as a function of temperature for irradiated PCL and SF/PCL composites. For all samples, the E' curve exhibits a plateau first and then decreases gradually after the glass transition of PCL component with temperature increasing. In the curves of E'', there is a peak between −70 and −50°C. The peak temperature can be used to represent the T_g of PCL component. For PCL, after irradiated with various doses, the difference of E' becomes more obvious at the temperature above glass transition. The E' increases with the irradiation dose up to 200 kGy and then decreases. The change of E'' has the same trend as that of E'. The peak of E'' is widened after irradiation and PCL200 exhibits a highest T_g. The T_g of PCL250 and PCL300 shift to lower temperature compared with PCL200. For SF/PCL composites, both of E' and E'' get a maximum at 150 kGy, and the peak of E'' is also widened after irradiation. However, it is noticeable that the E'' peak of irradiated SF/PCL composites shifts to higher temperature after irradiation, including 250 and 300 kGy.

Studies on irradiated PCL through dynamic viscoelastic method have already carried out by some researchers.[13,14,27] In their studies, discussions were mainly focused on the melt behavior of irradiated PCL. The glass transition behavior of irradiated PCL has been rarely discussed. Yang and Wu.[26] found that after irradiation treatment, the T_g of PCL component in chitin fiber-reinforced PCL composites increased with the fiber content, whereas the T_g of untreated composites remained almost at the same temperature. They suggested that crosslinking of PCL and strengthened interfacial interaction might be the reasons for the increasing of T_g. In Figure 5, the irradiation causes crosslinking reaction in PCL, which restricts the movement of PCL molecular chains and results in the increasing modulus and T_g. When the irradiation dose is more than 200 kGy, the

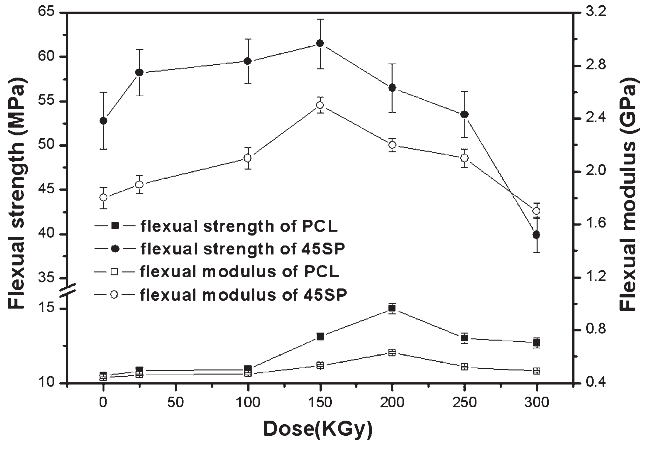

Figure 2 Effect of irradiation dose on the flexural strength and modulus of PCL and SF/PCL composites with 45% fiber content.

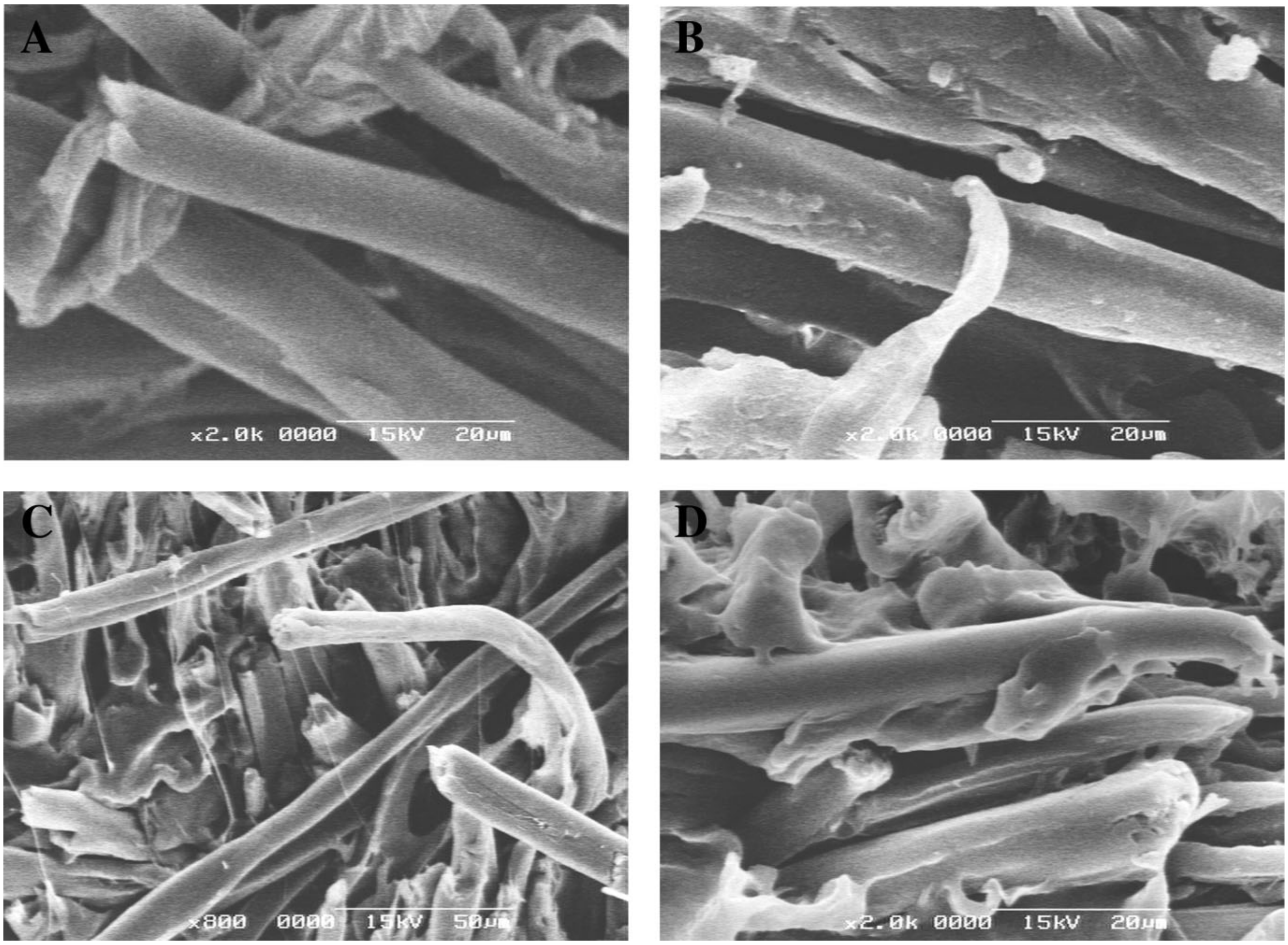

Figure 3 SEM photographs of tensile fractures of 45SP (A), 45SP25 (B), 45SP100 (C), and 45SP150 (D).

degradation of PCL causes a decrease in modulus and T_g. It is coincident with the results of the static flexural modulus of PCL, which also have peak values at 200 kGy. The crosslinking and degradation of PCL also widen the peak of E''. However, for irradiated SF/PCL composites, the crosslinking and degradation of PCL in composite cannot explain the increasing T_g at 250 and 300 kGy. Therefore, the interfacial bonding between fiber and matrix may be strengthened by irradiation, which restricts the movement of PCL molecular chains and can also widen the peak of E'' of SF/PCL composite.

EPR analysis

Compared with other samples being irradiated, the 45SP150 sample has better static and dynamic mechanical properties. Therefore, 45SP150 is chosen for further studying the effect of irradiation on the structure of composite. Figure 6 shows the EPR spectra of PCL150, 150 kGy-irradiated SF fiber, and 45SP150 measured immediately after irradiation. The EPR spectrum of 45SP is also shown. The 45SP sample also has an EPR signal, which may be assigned to the radicals formed during the melt-mixing process. It can be observed from Figure 6 that both PCL and SF fiber can produce free radicals after irradiation. However, there is no obvious difference between EPR signal of 150 kGy-irradiated SF fiber and that of 45SP150, indicating that most residual radicals in SF fiber and SF/PCL composite after irradiation are the same. There seems no radical interaction occurring between PCL and SF fiber immediately after irradiation. Figure 7 shows a decay of EPR signals after 1-month storage under ambient condition. Remarkably, the spectra shapes of 150 kGy-irradiated SF fiber and 45SP150 are different, suggesting that the residual radicals in them are not the same after the 1-month storage.

As reported by Ohrlander et al.[15] about the effect of electron beam irradiation on PCL, the PCL irradiated in air contained at least two kinds of free radicals, secondary alkylether radicals ($\text{—}CH_2CH_2CH^{\bullet}COO\text{—}$) and peroxy radicals. In their research, the different kinds of radical signals could be separated by varying the microwave used in ESR

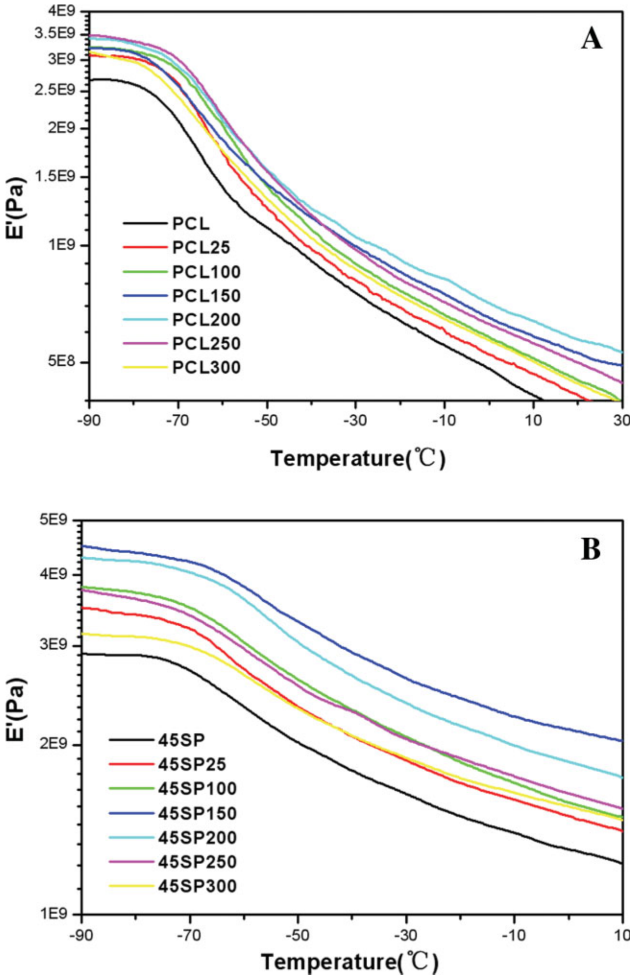

Figure 4 Effect of irradiation does on the storage modulus (E') of PCL (A) and SF/PCL composites with 45% fiber content (B). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

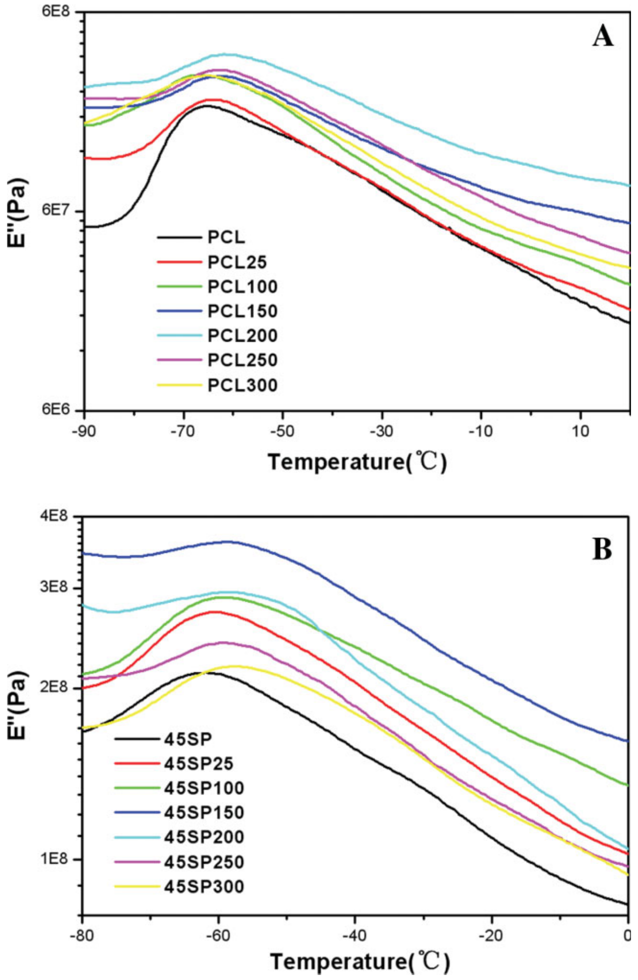

Figure 5 Effect of irradiation does on the loss modulus (E'') of PCL (A) and SF/PCL composites with 45% fiber content (B). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

analysis. At a low microwave power, the spectrum from secondary alkylether radicals was prominent, whereas at a high microwave power, the signal assigned to peroxy radicals was prominent. The EPR measurement in this article was performed at a lower microwave power of 0.2 mW in the presence of air. Therefore, the signals of secondary alkylether radicals are dominant and the signals of peroxyl radicals are not visible. In Figure 6, the EPR spectrum of PCL150 is similar to the signals observed by Filipczak et al.[16] The spectrum represents the radicals formed by abstraction of the hydrogen atom at α-site to an ester group. The hyperfine splitting may be assigned to two different radicals ($-CH_2CH_2CH^{\bullet}COO-$ or $-COOCH^{\bullet}CH_2CH_2-$). Filipczak et al.[16] also discussed the role of oxygen in irradiation of PCL. Oxygen can prevent crosslinking by reacting with alkyl radicals of PCL to form peroxyl radicals, which were usually more stable than alkyl radicals and were able to initiate a variety of

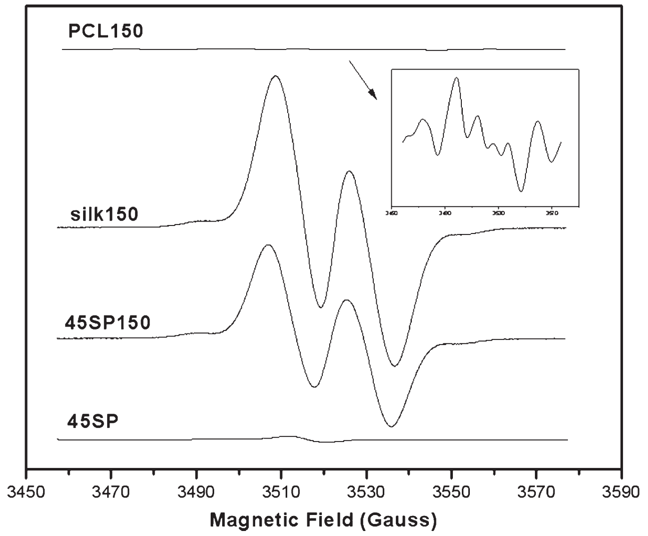

Figure 6 EPR spectra of PCL150, 150 kGy-irradiated silk fibroin fiber, 45SP150 immediately after irradiation and 45SP.

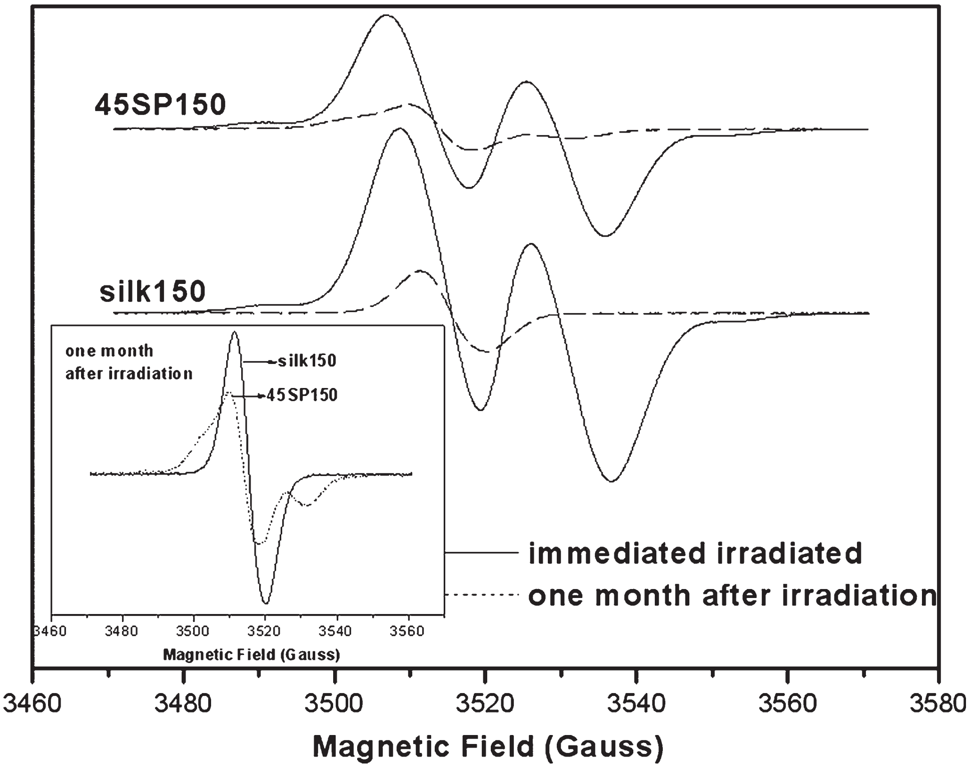

Figure 7 EPR spectra of silk fibroin fiber and SF/PCL composite measured directly after irradiation and after 1-month storage.

reaction pathways. So, the presence of these radicals makes it possible for irradiated PCL to react with the radicals formed in irradiated silk during the 1-month storage.

Several studies have investigated the EPR spectra of irradiated fibrous proteins such as silk and wool.[19,23,24,28] Mamedov et al.[23] summarized the mechanisms related to the processes by free radicals formed under the photolysis or radiation in protein. The formation of acyl radicals (—NHCH$^{\bullet}$CO—) and the split off hydrogen atom from C_{α}-atom could be found at 23°C. The EPR spectrum of 150 kGy-irradiated SF fiber in Figure 6 is similar to the results obtained by Liu et al.[19] The signals are assigned mainly to the acyl radicals formed in glycine and alanine,[28] which together account for more than 70% of the amino acid composition in the fiber.

As discussed earlier, both PCL and SF fiber can produce free radicals after irradiation. It is suggested that some transformations or reactions of these radicals may take place between PCL matrix and silk fiber during the 1-month storage. These transformations or reactions lead to the different shapes of EPR spectra between 150 kGy-irradiated SF fiber and 45SP150 measured after 1-month storage. As a result, the interfacial interaction may "graft" PCL matrix to SF fiber as shown in the SEM photographs and make the T_g of PCL component in SF/PCL composites shift to higher temperature after irradiation, as shown in Figure 5.

FTIR analysis

Figure 8 shows the FTIR spectra of PCL, SF fiber, and 45SP before and after irradiation. The bands of PCL in FTIR spectrum and their assignments were gathered by Elzein et al.[29] In Figure 8, the strong band around 1722 cm^{-1} is assigned to C=O stretching mode. The peaks around 1186 and 1163 cm^{-1} are correlated to OC—O stretching and symmetric COC stretching modes. The 1186 cm^{-1} band cannot be distinguished after irradiation and the band intensity is decreased in the region of 1160–1190 cm^{-1} with the irradiation dose, indicating the chain scission of OC—O caused by irradiation. For SF fiber, the doublets at 1230 and 1263 cm^{-1} are assigned to amide III, which are mainly due to the C—N—H in-plane bending and C—N stretching vibrations. The signal at 1263 cm^{-1} is associated with β-pleated-sheet conformation and the signal at 1230 cm^{-1} is associated with random-coil conformation.[30] There is a decrease in the signal intensity at 1230 and 1263 cm^{-1} after irradiation, indicating the scission of peptide chain. Both the chain scissions of PCL and SF fiber after irradiation can lead to the decrease of mechanical properties of SF/PCL composite. However,

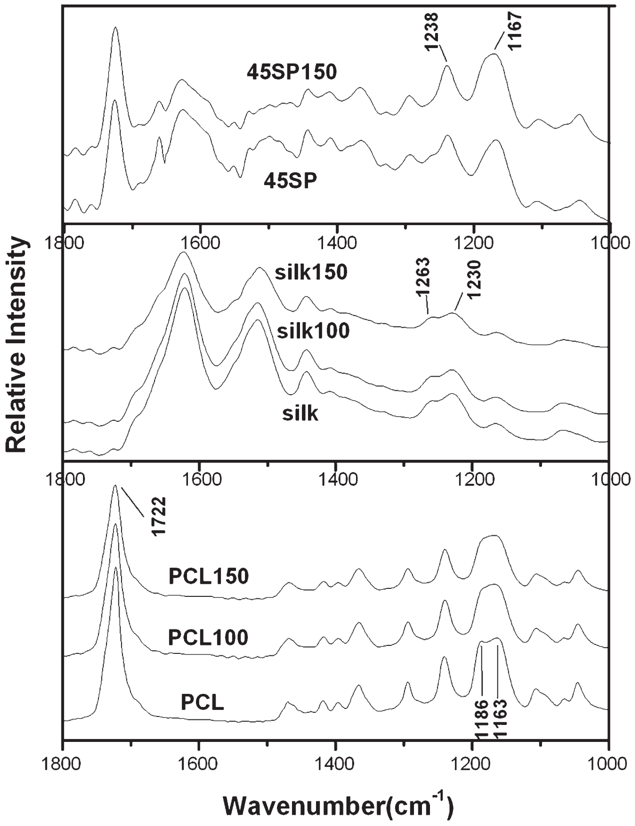

Figure 8 FTIR spectra of PCL, silk fibroin fiber, and 45SP before and after irradiation.

the chain scissions cannot be observed in the FTIR spectra of 45SP and 45SP150. The intensity of corresponding bands seems no obvious difference before and after irradiation. It is because that the signals of PCL at 1186 and 1163 cm^{-1} are overlapped by the signal of SF fiber at 1164 cm^{-1}, which is associated with the stretching of phenol group. And, the signals of SF fiber from 1230 to 1263 cm^{-1} are overlapped by the signal of PCL at 1240 cm^{-1} for the asymmetric stretching of COC.

CONCLUSIONS

During irradiation, the formation of free radicals and chain scissions occur in both PCL and SF fiber. Some transformations or reactions of radicals may take place between PCL matrix and SF fiber after irradiation as well as the degradation of PCL resin. In PCL, the intermolecular crosslinking and degradation of PCL widen the range of glass transfer temperature. However, in SF/PCL composite, the interfacial interaction between PCL and SF fiber is improved and enhances the static and dynamic mechanical properties of SF/PCL composite.

The authors thank Instrumental Analysis Center of Shanghai Jiao Tong University for the assistance with the measurements. "is revised as" The research was supported by Project Sponsored by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Minsistry. The authors also thank instrumental Analysis Center of Shanghai Jiao Tong University for the assistance with the measurements.

References

1. Cyras, V. P.; Kenny, J. M.; Vázquez, A. Polym Eng Sci 2001, 41, 1521.
2. Funabashi, M.; Kunioka, M. Green Chem 2003, 5, 591.
3. Teramoto, N.; Urata, K.; Ozawa, K.; Shibata, M. Polym Degrad Stab 2004, 86, 401.
4. Yang, A.; Wu, R. J Mater Sci Lett 2001, 20, 977.
5. Kumar, S.; Varma, I. K. Polym Plast Technol 2006, 45, 1219.
6. Kumar, S.; Varma, I. K. J Macromol Sci B 2006, 45, 153.
7. Pérez-Rigueiro, J.; Viney, C.; Llorca, J.; Elices, M. J Appl Polym Sci 1998, 70, 2439.
8. Shao, Z.; Vollrath, F. Nature 2002, 418, 741.
9. Freddi, G.; Mossotti, R.; Innocenti, R. J Biotechnol 2003, 106, 101.
10. Pérez-Rigueiro, J.; Elices, M.; Llorca, J.; Viney, C. J Appl Polym Sci 2002, 84, 1431.
11. Chmielewski, A. G.; Haji-Saeid, M.; Ahmed, S. Nucl Instrum Methods B 2005, 236, 44.
12. Darwis, D.; Mitomo, H.; Enjoji, T.; Yoshii, F. Makuuchi, K. J Appl Polym Sci 1998, 68, 581.
13. Yoshii, F.; Darwis, D.; Mitomo, H.; Makuuchi, K. Radiat Phys Chem 2000, 57, 417.
14. Darwis, D.; Nishimura, K.; Mitomo, H.; Yoshii, F. J Appl Polym Sci 1999, 74, 1815.
15. Ohrlander, M.; Erickson, R.; Palmgren, R.; Wirsén, A.; Albertsson, A.-C. Polymer 2000, 41, 1277.
16. Filipczak, K.; Wozniak, M.; Ulanski, P.; Olah, L.; Przybytniak, G.; Olkowski, R. M.; Lewandowska-Szumiel, M.; Rosiak, J. M. Macromol Biosci 2006, 6, 261.
17. Plikk, P.; Odelius, K.; Hakkarainen, M.; Albertsson, A. C. Biomaterials 2006, 27, 5335.
18. Oláh, L.; Filipczak, K.; Czvikovszky, T.; Czigány, T.; Borbás, L. Radiat Phys Chem 2007, 76, 1430.
19. Liu, J.; Shao, J.; Zheng, J. J Appl Polym Sci 2004, 91, 2028.
20. Shao, J.; Liu, J.; Chen, Z. Color Technol 2001, 117, 229.
21. Bashar, A. S.; Khan, M. A.; Ali, K. M. I. J Appl Polym Sci 1997, 63, 1703.
22. Bashar, A. S.; Khan, M. A.; Ali, K. M. I. Radiat Phys Chem 1995, 45, 753.
23. Mamedov, Sh. V.; Aktas, B.; Cantürk, M.; Aksakal, B.; Alekperov, V.; Bülbül, F.; Yilgin, R.; Aslanov, R. B. Biomaterials 2002, 23, 3405.
24. Wang, H.; Liu, R.; Tu, T.; Xie, L.; Sheng, K.; Chen, Y.; Tang, X. J Radiat Res 2004, 45, 77.
25. Li, W.; Qiao, X.; Sun, K.; Chen, X. J Appl Polym Sci 2008, 110, 134.
26. Yang, A.; Wu, R. J Appl Polym Sci 2002, 84, 486.
27. Mitomo, H.; Sasada, K.; Nishimura, K.; Yoshii, F.; Nagasawa, N. J Polym Environ 2004, 12, 95.
28. Shao, J.; Carr, C. M.; Rowlands, C. P.; Walton, J. J Text Inst 1999, 90, 459.
29. Elzein, T.; Nasser-Eddine, M.; Delaite, C.; Bistac, S.; Dumas, P. J Colloid Interface Sci 2004, 273, 381.
30. Shao, J.; Zheng, J.; Liu, J.; Carr, C. M. J Appl Polym Sci 1999, 2005, 96.