# Preparation and characterization of silk fibroin and methoxy poly(ethylene glycol)-*b*- poly(D,L-lactide) nanocomposite films

*Presented in The 2nd Progress in Advanced Materials: Micro/Nano Meterials and Applications*

*Yaowalak Srisuwan*[1], *Nualchai Kotsaeng*[1]
*Mangkorn Srisa-ard*[2], *Chaiyasit Sittiwet*[2]
*Yodthong Baimark*[2] *

## Abstract

Nanocomposite films of silk fibroin (SF) and methoxy poly(ethylene glycol)-*b*-poly(D,L-lactide) (MPEG-*b*-PDLL) were prepared by film casting of MPEG-*b*-PDLL nanoparticle suspension-SF aqueous solution. The nanoparticles were prepared in SF solution by modified-spontaneous emulsification solvent diffusion method without any surfactant. The interaction between SF and MPEG-*b*-PDLL films was studied by FT-IR spectroscopy. SEM results indicated that the nanoparticle sizes in films were in the range of 100 - 300 nm with spherical shape. Nanoporous with pore size in the range of 10-300 nm were observed throughout the nanocomposite films. The number and size of nanoporous increased when the MPEG-*b*-PDLL ratio was increased.

**Keywords:** Silk fibroin films, MPEG-*b*-PDLL, nanocomposite films, nanoporous films

## 1. Introduction

Silk fibroin (SF), one of the typical protein polymers spun by *Bombyx mori* silkworm, is a potential biomaterial for wide use in biological and biomedical fields due to its good biological compatibility as well as biodegradability (Altman *et al.*, 2003). Applications of SF in tissue engineering (Hofmann *et al.*, 2006) drug delivery (Chen et al., 2006) and wound healing (Roh et al., 2006) have been reported. The minimal inflammatory reactions *in vitro* and *in vivo* of SF film have been reported by Meinel et al. (Meinel et al., 2005). Poly(e-caprolactone-*co*-D,L-lactide) (PCLDLL) films containing SF microparticles have investigated (Kesenci *et al.*, 2001) The interactions between them were found. However, the SF films containing biodegradable nanoparticles have not been reported.

Methoxy poly(ethylene glycol)-*b*-poly (D,L-lactide) (MPEG-*b*-PDLL) diblock copolymers have been synthesized to attain versatile biodegradable polymers having more water-absorbing capacity because of the inclusion of hydrophilic MPEG block

[1]*Research Assistant, Department of Chemistry, Faculty of Science, Mahasarakham University*
[2]*Lecturer, Department of Chemistry, Faculty of Science, Mahasarakham University*
**Corresponding author, E-mail: yodthong.b@msu.ac.th*

within the relatively hydrophobic PDLL block (Lucke et al., 2000; Sun et al., 2004; Stefani et al., 2006). MPEG-b-PDLL have been used for the preparation of drug-loaded nanoparticles (Kim et al., 2005; Pierri and Avgoustakis, 2005; De Faria et al., 2005) with composed of the MPEG chains on the nanoparticle surfaces (Heald et al., 2002; Riley et al., 2003). Using of the modified-spontaneous emulsification solvent diffusion method (modified-SESD method) for preparing of surfactant-free MPEG-b-PDLL nanoparticles has been reported in our previous work (Baimark et al., 2007a). The use of a higher energy apparatus such as the homogenizer and sonicator in emulsion solvent evaporation method and high volume of water in dialysis method are avoided for this method. Hence, the large-scale preparation of the surfactant-free nanoparticles would be possible.

In the present study, we adapted the modified-SESD method to prepare colloidal nanoparticles of MPEG-*b*-PDLL in SF aqueous solution without any surfactant before film casting of this suspension-solution. Then, the SF/MPEG-*b*-PDLL nanocomposite film was obtained following this procedure. Preparation and characterization of SF nanocomposite films with different SF/MPEG-*b*-PDLL composite ratios were studied. The intermolecular interaction between SF and MPEG-*b*-PDLL were analyzed by FT-IR spectroscopy. Scanning electron microscopy was used to observe morphology of film surface and cross-section.

## 2. Experimental

### 2.1. Materials

Silk fibroin (SF) was prepared by a chemical degummed method and dissolved before dialysis and lyophilized, respectively. Cocoons from *B. mori* were degummed by boiling twice in 0.5% $Na_2CO_3$ solution at 98 - 100 °C for 30 min to remove sericin, then rinsed with distilled water and dried at room temperature. Degummed SF fibers were dissolved in the ternary solvent, $CaCl_2$-ethanol-water (mole ratio = 1:2:8), by stirring at 80 °C for 2 hrs. The resulting SF solution was then dialyzed (cellulose tube) for 3 days against distilled water. The final concentration after dialysis was adjusted to 1% (w/v) against distilled water.

Methoxy poly(ethylene glycol)-*b*-poly (D,L-lactide) (MPEG-*b*-PDLL) was prepared as described in our previous work (Baimark *et al.*, 2007a) by ring-opening polymerization of DLL at 130 °C for 24 hrs using MPEG with molecular weight of 5,000 g/mol (Fluka, Germany) and stannous octoate (95%, Sigma, USA) as the initiating agents. The stannous octoacte concentration was 0.02 mol%. As-polymerized MPEG-*b*-PDLL was purified by precipitation in *n*-hexane from chloroform solution before dried *in vacuo* at room temperature until constant weight. Number-average molecular weight and polydispersity of the MPEG-*b*-PDLL were determined from GPC as 73,600 and 1.88, respectively. All solvents and non-solvents, the analytical grade were used.

### 2.2. Preparation of SF nanocomposite films

The SF nanocomposite films with different SF/MPEG-*b*-PDLL composite ratios were prepared by film casting of SF aqueous solutions containing surfactant-free MPEG-*b*-PDLL nanoparticles. The colloidal nanoparticles were prepared by modified-SESD method in SF aqoueous solution. Briefly, the MPEG-*b*-PDLL solution in mixture organic solvent of acetone/ethanol [3/3 (v/v)] was added

drop-wise into SF solution with stirring at 600 rpm. The organic solvents were evaporated in fume hood for 6 hrs. Then, colloidal nanoparticles of MPEG-*b*-PDLL in SF solution was obtained before poured on Petri dish and dried at 40 °C for 24 hrs. The dried films were treated with 90% (v/v) methanol aqueous solution for 1 hr before dried *in vacuo* at room temperature for one week. The SF/MPEG-*b*-PDLL composite ratios of the SF nanocomposite films were summarized in Table 1.

### 2.3. Characterization

FT-IR spectra were collected by Fourier transform infrared (FT-IR) spectroscopy using a Perkin-Elmer Spectrum GX FTIR spectrophotometer with air as the reference. The resolution of 4 $cm^{-1}$ and 32 scans were chosen in this work.

Film morphology was investigated by scanning electron microscopy (SEM) using JEOL JSM-6460LV SEM. The film was fractured in liquid nitrogen and coated with gold for enhanced conductivity before scan.

## 3. Results and discussion

### 3.1. FT-IR spectra

The structures of SF, MPEG-*b*-PDLL and SF nanocomposite films were characterized, and the SF conformation transition of SF nanocomposite films was determined by FT-IR spectroscopy. The position of absorption bands indicated the conformation of SF. Figure 1 shows the FT-IR spectra of SF, MPEG-*b*-PDLL and SF nanocomposite films. The absorption bands of SF film in Figure 1(a) at 1681 (amide I), 1573 (amide II) and 1234 $cm^{-1}$ (amide III) were assigned to the random coil structure of SF film. The absorption bands at 1615 (amide I) and 1521 $cm^{-1}$ (amide II) were indicated *b*-sheet structure of SF film (Wu et al., 2006). The splitting results of the amide bands indicated that the random coil and $\beta$-sheet structures were coexisted in the SF film after the methanol solution treatment. The MPEG-*b*-PDLL film was prepared by film casting of MPEG-*b*-PDLL solution in dichloromethane. The FT-IR spectrum of MPEG-*b*-PDLL film in Figure 1 (e) showed strong absorption band at 1760 $cm^{-1}$, attributed to the carbonyl stretching. The spectra of the SF nanocomposite films with SF/MPEG-*b*-PDLL composite ratios of 20/1, 20/2 and 20/3 (w/w) in Figures 1 (b) – (d), respectively showed the characteristic bands of both SF and MPEG-*b*-PDLL components. In parallel with hypothesis, the intensity of carbonyl bands of the SF nanocomposite films increased when the MPEG-*b*-PDLL ratio was increased.

**Table 1** Composite ratios of SF/MPEG-*b*-PDLL nanocomposite films.

| SF/MPEG-*b*-PDLL nanocomposite films (w/w) | 1% (w/v) SF solution (mL) | MPEG-*b*-PDLL [a] (mg) |
|---|---|---|
| 20/0 | 20 | - |
| 20/1 | 20 | 0.01 |
| 20/2 | 20 | 0.02 |
| 20/3 | 20 | 0.03 |

[a] amount of MPEG-*b*-PDLL dissolved in 2 mL [3/3 (v/v)] acetone/ethanol mixture solvent.

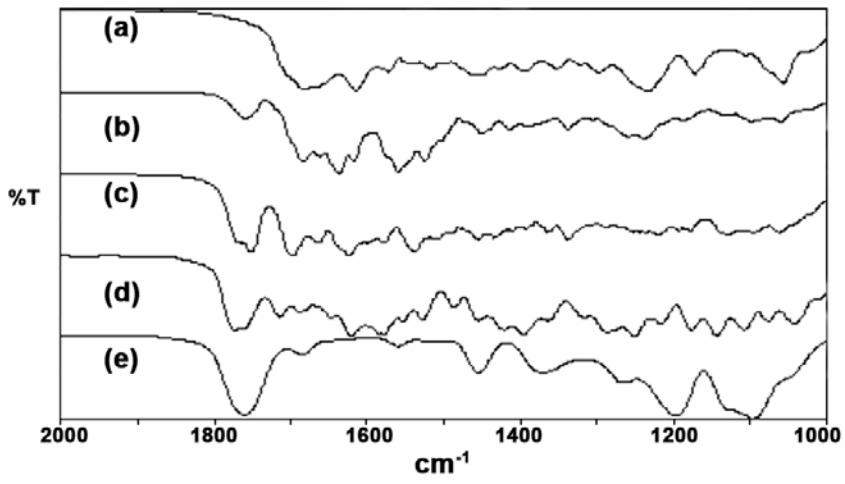


**Figure 1.** FT-IR spectra of (a) SF, (b) 20/1 SF nanocomposite, (c) 20/2 SF nanocomposite, (d) 20/3 SF nanocomposite and (e) MPEG-*b*-PDLL films.

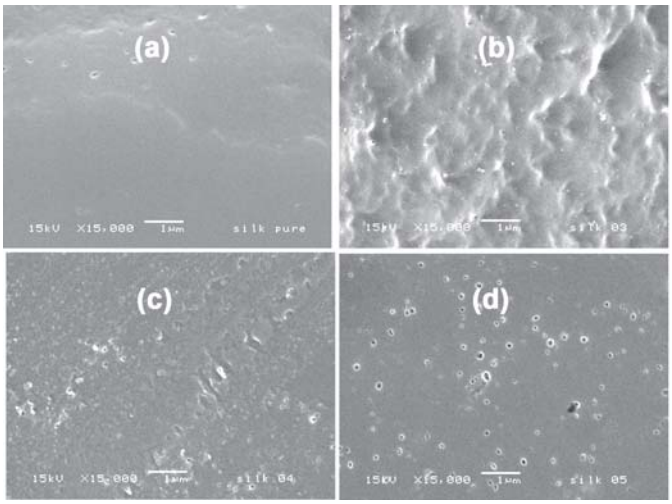


**Figure 2.** SEM images of film surfaces of (a) SF, (b) 20/1 SF nanocomposite, (c) 20/2 SF nanocomposite and (d) 20/3 SF nanocomposite films.

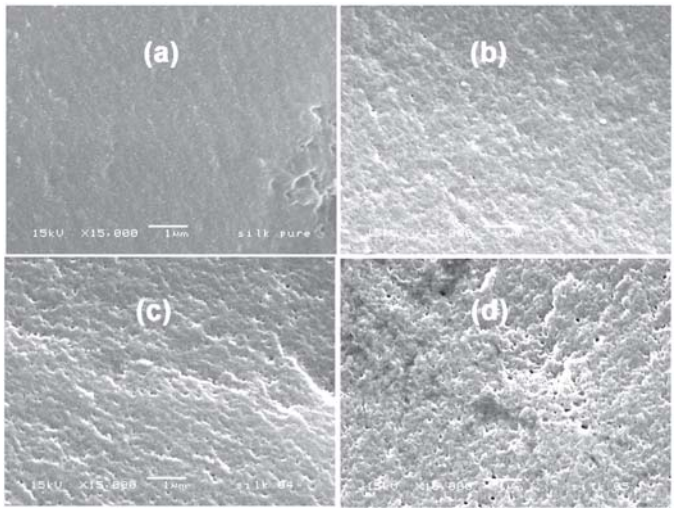


**Figure 3.** SEM images of film cross-sections of (a) SF, (b) 20/1 SF nanocomposite, (c) 20/2 SF nanocomposite and (d) 20/3 SF nanocomposite films.

The amide I and II bands for the SF nanocomposite films were shifted to higher wave number [Figures 1(a) – 1(d)] indicated that the specific intermolecular interaction between SF and MPEG-*b*-PDLL were existed. It was proposed that the formation of nanoparticles could induce the partial transformation of SF to a random coil conformation.

### 3.2. Film morphology

Thickness of the SF and its nanocomposite films was determined by SEM analysis. It was found to be in the range of 30 – 60 mm. Figure 2 shows the film surfaces morphology of SF and its composite films. The MPEG-*b*-PDLL nanoparticles can be detected on the film surfaces of SF nanocomposite films with approximately 100 nm in sizes. The nanopores on film surfaces were measured for SF nanocomposite films with the nanopore sizes in the range of 50 – 300 nm. This due to self-condensed and nanophase separated of the MPEG-*b*-PDLL nanoparticles from the SF matrix during the drying process (Baimark et al., 2007b). The number and size of nanopores increased as increasing the MPEG-*b*-PDLL ratios.

Figure 3 shows the film cross-sections morphology of SF and its composite films. The film cross-sections of SF nanocomposite films were rougher than its surfaces. The MPEG-*b*-PDLL nanoparticles and nanopores can be clearly observed that dispersed into the SF film in the ranges of 100 – 300 and 10 – 300 nm with spherical shape. Figure 4 shows expanded SEM image of 20/3 SF nanocomposite film suggested that the SF nanocomposite films contain interconnected nanopores throughout the film. This indicated that the SF nanocomposite films were nanoporous films.

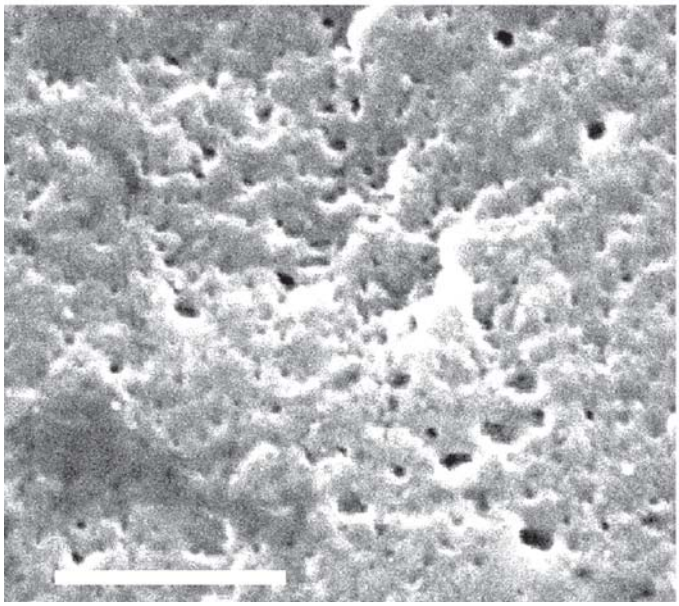

**Figure 4.** Expanded SEM image of film cross-sections of 20/3 SF nanocomposite films (bar = 1,000 nm).

## 4. Conclusions

The SF nanocomposite films with different SF/MPEG-*b*-PDLL composite ratios were successfully prepared by film casting of the surfactant-free MPEG-*b*-PDLL nanoparticles suspended in SF aqueous solution. The intermolecular interactions between SF matrix and MPEG-*b*-PDLL nanoparticles were investigated from FT-IR results. The SEM images showed the nanoparticles with spherical shape on film surfaces and cross-sections and diameter of particle in the range of 100-300 nm. The SF nanocomposite films show nanoporous structure with the pore size in the range of 10-300 nm. The number and size of nanoparticles and nanopores increased when the MPEG-*b*-PDLL ratio was increased. These nanoparticle-loaded SF nanocomposite films with nanoporous structure might be of interested for biomedical and pharmaceutical applications.

## Acknowledgment

The authors would like to acknowledge the Research Development and Support Unit, Mahasarakham University, fiscal year 2008 for financial support.

## References

Altman, G.H., Diaz, F., Jakuba, C., Calabro, T., Horan, R.L., Chen, J., Lu, H., Richmond, J. and Kaplan, D.L. 2003. Silk-based biomaterials. **Biomaterials** 24(3): 401- 416.

Baimark, Y., Srisa-ard, M., Threeprom, J. and Narkkong, N. 2007a. Preparation of nanoparticle colloids of methoxy poly(ethylene glycol)-*b*-poly(D,L-lactide): effects of surfactant and organic solvent **Colloid Polym Sci** 285(13): 1521-1525.

Baimark, Y., Niamsa, N., Morakot, N., Threeprom, J. and Srisuwan, Y. 2007b. Preparation and morphology study of biodegradable chitosan/ methoxy poly(ethylene glycol)-*b*-poly (ε-caprolactone) nanocomposite films. **Int J Polym Anal Charact** 12(6): 457-467.

Chen, G., Zhou, P., Mei, N., Chen, X., Shao, Z.Z., Pan, L. and Wu, C. 2004. Silk fibroin modified porous poly(ε-caprolactone) scaffold for human fibroblast culture in vitro. **J Mater Sci Mater Med** 15(6): 671-677.

De Faria, T.J., De Campos, A.M. and Senna, E.L. Preparation and characterization of poly (D,L-lactide) (PLA) and poly(D,L-lactide)-poly(ethylene glycol) (PLA-PEG) nanocapsules containing antitumoral agent methotrexate. 2005. **Macromol Symp** 229(1): 228-233.

Heald, C.R., Stolnik, S., Kujawinski, K.S., De Matteis, C., Garnett, M.C., Illum, L., Davis, S.S., Purkiss, S.C., Barlow, R.J. and Gellert, P.R. 2002. Poly(lactic acid)-poly(ethylene oxide) (PLA-PEG) nanoparticles: NMR studies of the central solidlike PLA core and the liquid PEG corona. **Langmuir** 18(9): 3669-3675.

Hofmann, S., Wong Po Foo, C.T., Rossetti, F., Textor, M., Vunjak-Novakovic, G., Kaplan, D.L., Merkel, H.P. and Meinel, L. 2006. Silk fibroin as an organic polymer for controlled drug delivery. **J Control Release** 111(1-2): 219-227.

Kesenci, K., Motta, A., Fambri, L. and Migliaresi, C. 2001. **Poly(epsilon-caprolactone-co-D,L-lactide)/silk fibroin composite materials: preparation and characterization. J Biomater Sci Polym Edn** 2(5): 337-351.

Kim, S.Y., Lee, Y.M. and Kang, J.S. J. 2005. Indomethacin-loaded methoxy poly(ethylene glycol)/poly(D,L-lactide) amphiphilic diblock copolymeric nanospheres: Pharmacokinetic and toxicity studies in rodents. **J Biomed Mater Res** 74A(4): 581-590.

Lucke, A., TeBmar, J., Schnell, E., Schmeer, G. and Gopferich, A. 2000. Biodegradable poly(D,L-lactic acid)-poly(ethylene glycol)-monomethyl ether diblock copolymers:structures and surface properties relevant to their use as biomaterials. **Biomaterials** 21(23): 2361-2370.

Meinel, L., Hofmann, S., Karageorgiou, V., Kirker-Head, C., McCool, J., Gronowicz, G., Zichner, L., Langer, R., Vunjak-Novakovic G. and Kaplan, D.L. 2005. The inflammatory responses to silk films in vitro and in vivo. **Biomaterials** 26(2): 147-155.

Pierri, E. and Avgoustakis, K. 2005. Poly(lactide)-poly(ethylene glycol) micelles as a carrier for griseofulvin. **J Biomed Mater Res** 75A(3): 639-647.

Riley, T., Heald, C.R., Stolnik, S., Garnett, M.C., Illum, L. and Davis, S.S. 2003. Core-shell structure of PLA-PEG nanoparticles used for drug delivery. **Langmuir** 19(20): 8428-8435.

Roh, D.-H., Kang, S.-Y., Kim, J.-Y., Kwon, Y.-B. L, K.-G. Park, Y.-H. Baek, R.-M. Heo, C.Y. Choe, J. and Lee, J.-H.. 2006. Wound healing effect of silk fibroin/alginate blended sponge in full thickness skin defect of rat. **J Mater Sci Mater Med** 17(6): 547-552.

Stefani, M., Coudane, J. and Vert, M. 2006. Effects of polymerization conditions on the in vitro hydrolytic degradation of plaques of poly (dl-lactic acid-*block*-ethylene glycol) diblock copolymers. **Polym Degrad Stab** 91(12): 2853-2859.

Sun, J., Hong, Z., Yang, L., Tang, Z., Chen, X. and Jing, X. 2004. Study on crystalline morphology of poly(L-lactide)-poly(ethylene glycol) diblock copoly Study on crystalline morphology of poly(L-lactide)-poly(ethylene glycol) diblock copolymer. **Polymer** 45(17): 5969-5977.

Wu, Y., Shen, Q. and Hu, S. 2006. Direct electrochemistry and electrocatalysis of heme-proteins in regenerated silk fibroin film. **Anal Chim Acta** 558(1-2): 179-186.