

Structural Characteristics and Properties of Silk Fibroin/Polyurethane Blend Films

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In this paper, silk fibroin (SF)/polyurethane (PU) blend films were fabricated to develop a new biomaterial for biomedical applications. These blend films were prepared using formic acid as a cosolvent, and structural characteristics and properties of blend films were investigated. FTIR results suggested that there was no specific interaction between SF and PU, implying molecular immiscibility in SF/PU blend films. Furthermore, it was revealed by XRD method that the crystalline region of blend components was not perturbed by counterpart polymers. The degree of phase separation of SF/PU blend films was diminished by increasing PU content in blend. Especially, the blend with 70% content of PU showed no evidence of macro-phase separation in SEM observation. However, SF/PU blend (70/30) was revealed to be phase-separated in a lower dimension confirmed by DMTA measurement. TGA result showed that thermal decomposition temperature of blend film was slightly decreased compared to those of SF and PU polymer itself. Though mechanical properties of SF/PU blend films were not good enough due to the solvent, blood compatibility of PU can be enhanced markedly by mixing with SF for SF/PU blend film.

Key words: Silk fibroin, Polyurethane, Blend film, Phase separation, Mechanical properties, Blood compatibility

Introduction

Silk fibroin (SF), the typical natural macromolecules spun by *Bombyx mori* silkworm, has been mainly used as textile fibers with excellent performance. Recently, several researchers have investigated SF as one of promising resources of biotechnology and biomedical materials due to its unique properties including good biocompatibility (Sakabe *et al.*, 1989), good oxygen permeability (Minoura *et al.*, 1990), minimal inflammatory reaction (Santin *et al.*, 1999), good enzyme immobilization ability (Yoshimizu and Asakura, 1990), and high cell attaching and growing ability (Minoura *et al.*, 1995).

However, SF films in the dry state, being very brittle, are not suitable for use by themselves. Therefore, many studies have been performed to improve physical properties of SF film using blend technique. Several researchers reported that physical properties of SF film could be enhanced by mixing with other synthetic or natural polymers, such as poly(vinyl alcohol) (Um and Park, 1998), sodium alginate (Liang and Hirabayashi, 1992), chitosan (Kweon *et al.*, 2001), and so on.

Polyurethanes (PU) have been utilized in the field of biomedical devices including artificial heart (Park *et al.*, 1999), intravascular devices (Francois *et al.*, 1996) and cartilage and bone repair (Radder *et al.*, 1996) due to their outstanding mechanical and chemical properties. Though they, in particular, have been considered as one of common blood contacting biomedical materials due to their relatively good blood compatibility, attempts have been made to improve blood compatibility in long term applications (Han *et al.*, 1995; Park *et al.*, 1999).

Polymer blend technique is one of the simplest and most efficient tools to tailor various properties without

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much economic and technical consideration. Therefore, it has been used to produce materials with proper properties necessary for biomedical and biotechnological application field. Especially, blood compatibility of one polymer component can be improved by blending with other bio-compatible polymers. Therefore, in this study, the structure and properties of SF/PU blend films with various blend ratios will be investigated for examining the feasibility of their applications as a new biomedical material.

Materials and Methods

Preparation

SF was obtained by degumming *Bombyx mori* cocoons. The cocoons were degummed twice with a marseillus soap 0.5% (o. w. f.) and sodium carbonate 0.3% (o. w. f.) solution at 100°C for 1 hr, and rinsed thoroughly in warm distilled water.

SF was first dissolved in a ternary solvent system of $\text{CaCl}_2/\text{H}_2\text{O}/\text{EtOH}$ solution (1/8/2 mole ratio) for 30 min at 85°C. Aqueous SF solution was obtained by dialysis of dissolved SF solution in a cellulose tube (molecular cutoff = 12,000 ~ 14,000) against distilled water for 4 days at room temperature. Then, aqueous SF solution was cast onto polystyrene plate and dried at room temperature to prepare regenerated SF.

PU beads (Pellethane 2363-80AE, Dow Chemical Co), used in this study, consist of hard segment, containing MDI (4-4' methylene diphenyl diisocyanate) and BDO (1-4-butanediol), and soft segment containing PTMO (polytetramethylene oxide). The chemical structure of Pellethane is depicted in Fig. 1.

Formic acid solutions of SF/PU blend were prepared from SF and PU, separately, being dissolved in 98% formic acid. SF/PU blend solutions were cast onto a poly-

styrene plate and allowing the solvent to evaporate at room temperature to produce SF/PU blend films. The thickness of these films was controlled ca. 50 μm . The solvent was eliminated by storing the films in vacuum oven for several days.

Measurements

FTIR spectra were obtained with Midac M series spectrometer (USA) in the spectral region of 4000~400 cm^{-1} . All samples were measured by transmittance method. For film measurement, the thickness of specimens was controlled ca. 5 μm .

X-ray diffraction (XRD) analysis was performed on Small-Angle X-ray Scattering with General Area Detector Diffraction System (GADDS, Bruker-Axs, Germany) using $\text{Cu K}\alpha$ radiation. Irradiation conditions were 45 kV and 40 mA.

Dynamic mechanical response of samples was monitored using Rheometric Scientific DMTA Mark IV (USA) in the tensile mode to evaluate T_g of SF/PU blends. Rectangular samples, $30 \times 5 \times 0.05$ mm, were scanned isochronally at a heating rate of 4°C/min. The frequency oscillation was adjusted to 1 Hz. All sample were stored in silica gel container before measurement to exclude the effect of water.

Thermogravimetric analysis was run under the flow of nitrogen gas at a scanning speed of 15°C/min using Rheometric Scientific TGA 1000 (USA). All samples were stored in silica gel container before measurement to exclude the effect of water.

Stress-strain curves were obtained using Minimat (Rheometric Scientific, USA). The experiments were performed with 200 N of load cell and 1 mm/min of tensile speed. $5 \times 20 \times 0.05$ mm of sample dimension was used and all samples were measured 10 times for each sample.

The fractured cross sectional surface of SF/PU blend

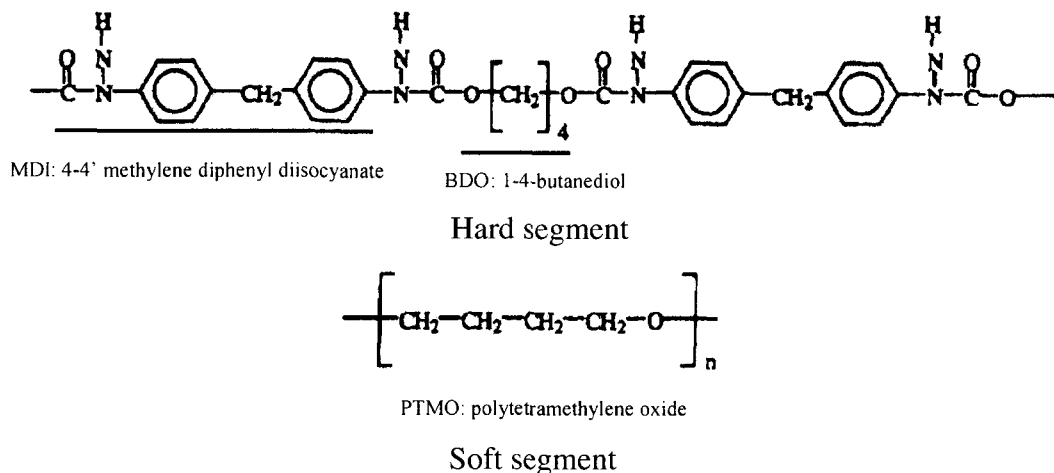


Fig. 1. Chemical structure of Pellethane 2363-80A.

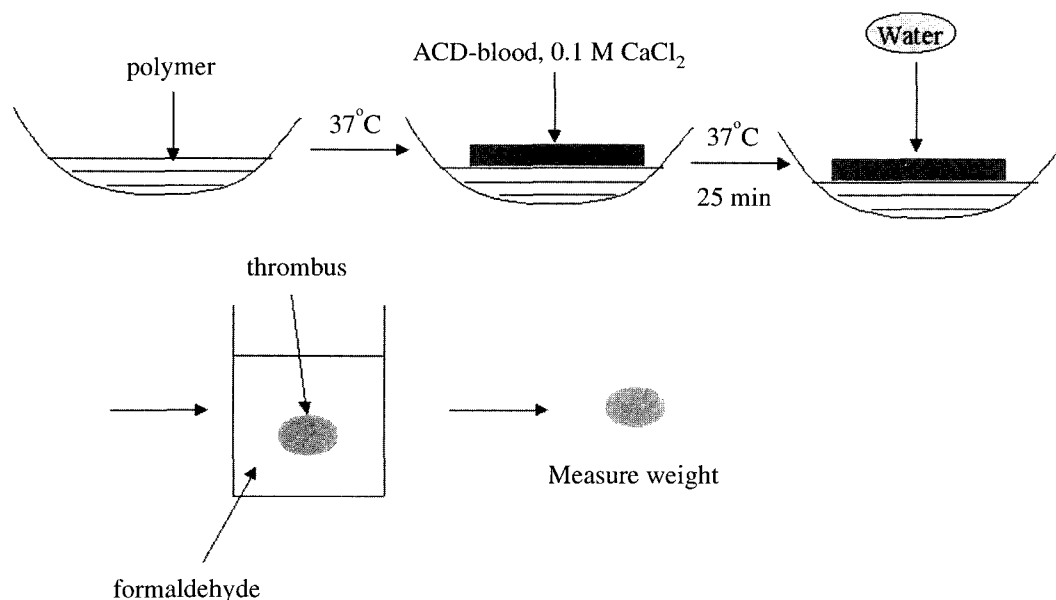


Fig. 2. Scheme of blood compatibility test for SF/PU blend films.

films were observed by scanning electron microscope (JSM-5410LV, JEOL, Japan) after gold coating.

Imai-Nose method (Imai and Nose, 1972) were utilized to evaluate blood compatibility of SF/PU blend films. As illustrated in Fig. 2, SF/PU blend films were coated on a glass dish. Then, the blood coagulation was started by adding 200 μ l of blood containing ACD (acid-citrate dextrose) and 50 μ l of 0.1 M calcium chloride into SF/PU coated glass dish. Then, the blood coagulation was finished by adding 15 ml of water into the dish after 25 min. The blood coagulant was separated from the dish and dipped into glutaraldehyde solution. After that, the blood coagulant was dried in vacuum oven and then its weight was measured. Finally, Relative % value of amount of blood coagulant for SF/PU samples against that for untreated (uncoated dish) was utilized as a barometer for blood compatibility.

Results and Discussion

Structural characteristics

FTIR spectroscopy is a useful tool for determining the conformation of SF and evaluating the miscibility in polymer blends. The promise of FTIR spectroscopic method is that the miscibility with a second polymer will perturb the local environment of oscillating dipole, and causing a shift and change in intensity of characteristic absorption. The way, in which FTIR spectrum is perturbed, may therefore be informative about the molecular environment, and in particular for the presence of specific interactions between functionalities of polymers. Accordingly, FTIR spectroscopy of SF blends was used to investigate structural characteristics and specific interactions between SF and PU.

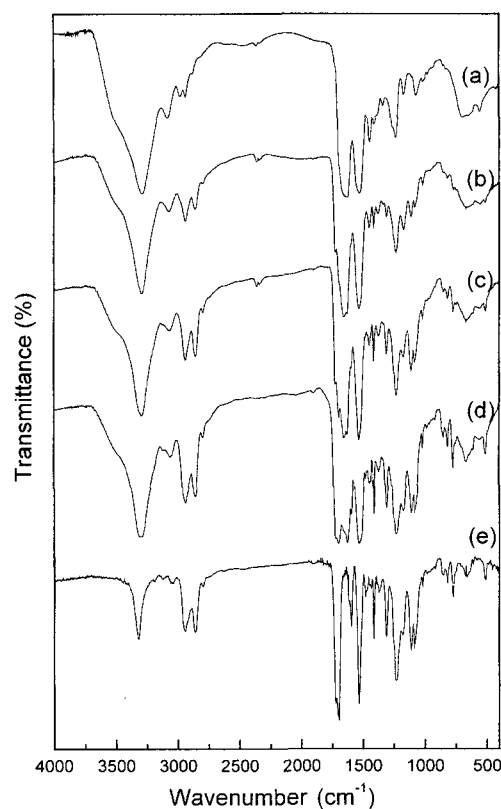


Fig. 3. FTIR spectra of SF/PU blend films with various blend ratios; (a) 100/0, (b) 70/30, (c) 50/50, (d) 30/70 and (e) 0/100.

copy of SF blends was used to investigate structural characteristics and specific interactions between SF and PU.

Fig. 3 shows FTIR spectra of SF/PU blend films with

various blend ratios. SF film showed absorption peaks at 1628 (amide I), 1533 (amide II), and 1235 cm^{-1} (amide III) attributed to a β -sheet conformation (Miyazawa and Blout, 1961). This indicates that the β -sheet conformation was induced by formic acid casting same as the result reported by Um *et al.* (2001). PU film exhibited absorption bands at 3320 (NH stretching), 1700 (CO stretching), 1591 (benzene ring stretching), and 1109 cm^{-1} (COC stretching), which are characteristic peaks of Pellethane (Fare *et al.*, 1999). In case of SF/PU blend films, the mixed spectra of SF and PU were obtained without any changes in position as well as intensity of absorption bands. The miscibility of polymer blend is generally attributed to specific interaction between blend components such as hydrogen bonding, causing a peak shift or presence of new peak (Andrew, 1992). Therefore, it can be said that there is no specific interaction between SF and PU, suggesting molecular immiscibility in SF/PU blend films. Furthermore, Fig. 3 shows that the β -sheet conformation of SF was not destroyed by mixing with PU molecules. This indicates PU molecules is not miscible with SF enough to perturb molecular arrangement of SF.

XRD curves of SF/PU blend films are shown in Fig. 4. SF film exhibits three diffraction peaks at 8.8, 19.4, and 23.5°, corresponding to the β -sheet crystalline spacing of

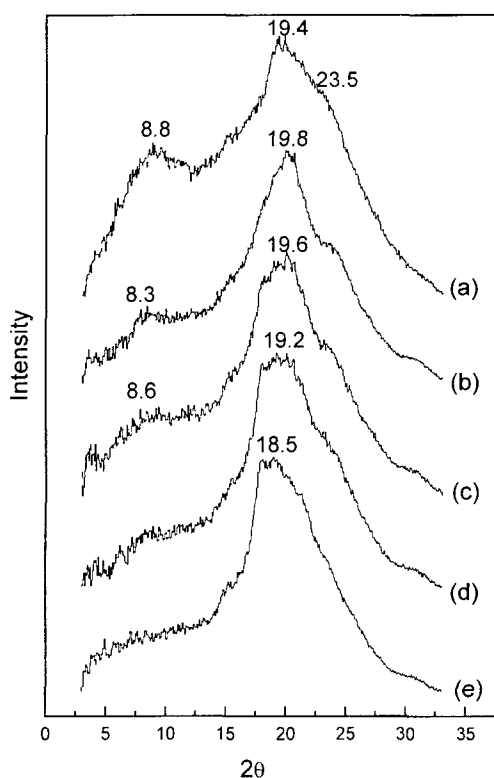
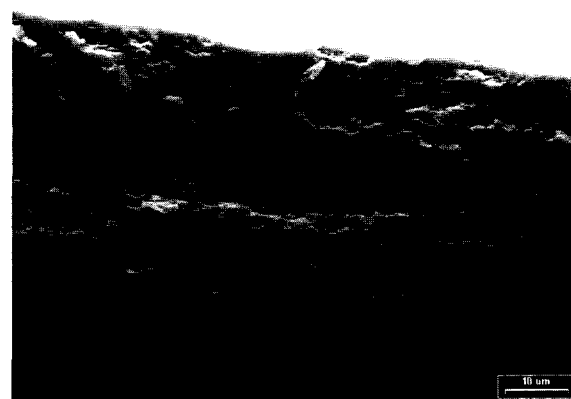
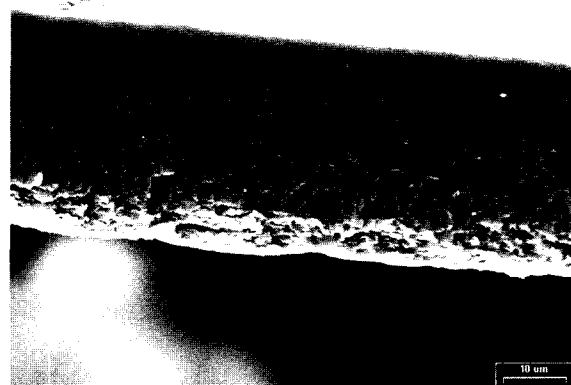


Fig. 4. X-ray diffractograms of SF/PU blend films with various blend ratios; (a) 100/0, (b) 70/30, (c) 50/50, (d) 30/70 and (e) 0/100.

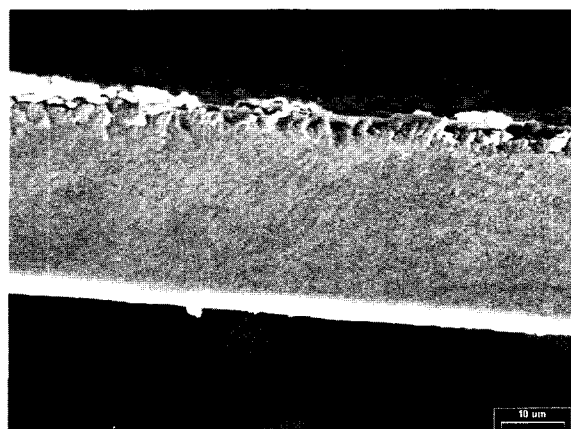
10.0, 4.6, and 3.8 Å, respectively (Magoshi *et al.*, 1979). On the other hand, PU shows a diffraction at $2\theta = 18.5^\circ$ due to crystalline spacing of hard segment block consisted of MDI and BDO (Quay *et al.*, 1990). In case of SF/PU blend films, they show overlap diffraction peaks of original components. This implies that the blend components



(a)



(b)



(c)

Fig. 5. SEM micrographs of SF/PU blend films with various blend ratios; (a) 70/30, (b) 50/50 and (c) 30/70.

can not penetrate a crystalline region of counterpart polymer, resulting in no disruption of crystalline arrangement. This result is consistent with that of FTIR spectroscopy.

Miscibility

The phase behavior, one of important research subjects in polymer blend, is significantly affected by the miscibility of polymer blend. The phase morphology of SF/PU blend films with different blend composition was examined by SEM and the results are shown in Fig. 5. The phase separation is occurred in 70/30 SF/PU blend film. However, as the composition of PU increases, the degree of phase separation decreases and finally, blend film containing 70% of PU component shows no phase separation morphology. This result means that miscibility of SF/PU blend films is improved by an increase of PU content.

A number and sharpness of T_g peak in polymer blend is a criterion for the determination of phase separation. Therefore, the measurement of glass transition temperature (T_g) is used as a common method for determining the miscibility in polymer-polymer blends. For example, the number of T_g s in blends imply the number of separated phase. One T_g in blend means the blend polymer system is homogeneous phase. Sharpness of T_g peak reflects the degree of homogeneity in the phase. Though electron microscopy has been used to determine the phase present in a blend, it bears an intrinsic limitation in terms of sensitivity of detectable phase domain size, approximately larger than 100 nm. However, the evaluation of T_g through dynamic mechanical measurements is sensitive enough to investigate the phase behavior even at 10 nm (Garton, 1992).

Therefore, dynamic mechanical measurements of SF/PU blend films are performed to examine a molecular miscibility of SF/PU blends at lower dimension scale. Fig.

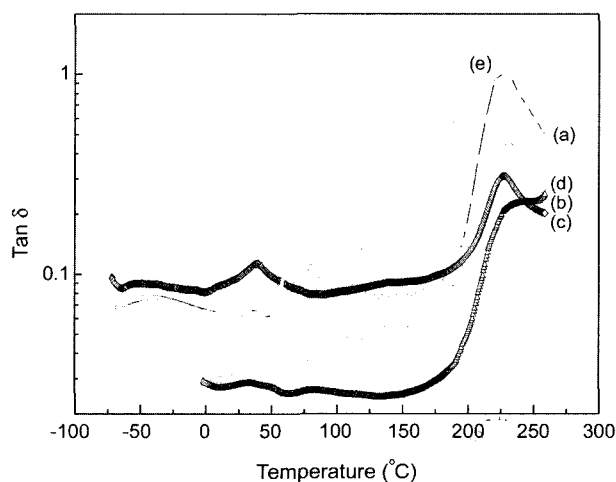


Fig. 6. Tan δ of SF/PU blend films with various blend ratios; (a) 100/0, (b) 70/30, (c) 50/50, (d) 30/70 and (e) 0/100.

6 displayed damping peaks of SF/PU blends films. SF and PU showed tan δ peak at about 225 and 60°C, respectively, corresponding to a glass transition of each polymer. In case of SF/PU blend films, they had two relaxation peaks in entire composition ranges examined, implying molecular immiscibility of these blends. It is interesting to note that even SF/PU blend film containing 30% PU content, which showed no phase separation in SEM observation, exhibited a phase separation in the lower dimension, being immiscible molecularly.

Based on above results, though some SF/PU blend showed one phase in SEM observation, most blend films were revealed to be immiscible. The immiscibility of SF/PU blends might be resulted from an absence of specific interaction as appeared in FTIR study.

Thermal degradation behavior

Thermal degradation behavior of polymer blends has been attracted because this serves for a better understanding of thermal stability and the structural characteristics of blends. For this, thermogravimetric analysis (TGA) was performed and differential thermogravimetric (DTG) curves of SF/PU blend films (Fig. 7) was obtained to investigate thermal decomposition behavior in detail.

Maximum thermal decomposition temperature (T_{max}) was

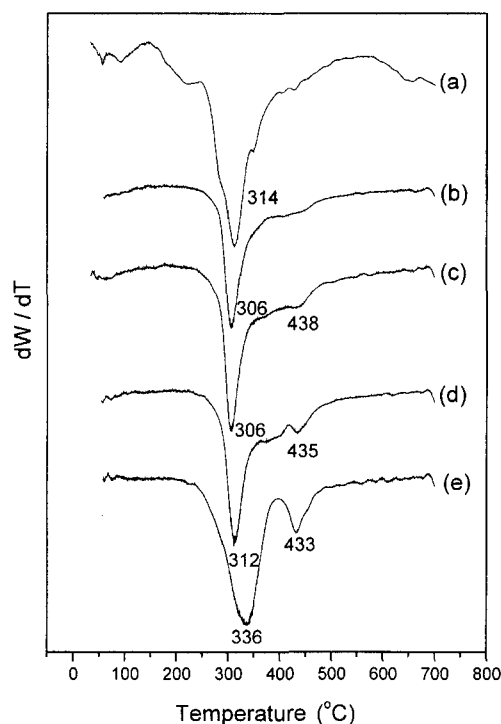


Fig. 7. Derivative TGA curves of SF/PU blend films with various blend ratios; (a) 100/0, (b) 70/30, (c) 50/50, (d) 30/70 and (e) 0/100.

measured from peak of DTG curves. SF showed a T_{\max} at 314°C attributed to thermal decomposition of the β -sheet crystalline. On the contrary, PU exhibited two T_{\max} s at 336°C and 433°C due to thermal decomposition of hard and soft segment, respectively (Wang *et al.*, 1999). SF/PU blend films showed two thermal decomposition peaks in entire composition ranges. Main thermal decomposition of blends occurred at around 306 ~ 312°C corresponding to overlap decomposition peak of SF and PU. Interestingly, a main T_{\max} of blend is lower than that of either individual component, suggesting that thermal decomposition of SF as well as hard segment of PU are accelerated by counterpart polymers.

When polymer blend is subjected to thermal degradation conditions, there is a possibility that any of polymer components or their degradation products will interact with each other, thus modifying the degradation behavior with respect to that of pure polymers. In some cases, the polymer in a blend is thermally stabilized (thermal decomposition is retarded) by interactions between their components, while in opposite cases, the degradation of blend component is accelerated. (Hale and Bair, 1997). The example of the former includes PVC/PS, the latter, PVC/PMMA, P_{α} PMS/PS and PVA/PVC blend. There are also examples of polymer blends including PS/PMMA that show no evidence of degradation or stabilization.

Therefore, the change in T_{\max} results from the presence of interaction between blend components when the polymers are thermally decomposed. Um *et al.* (2000) suggested that SF retarded the degradation of Poly(2-hydroxyethyl methacrylate) in blends due to interaction between blend components. Accordingly, the fact that T_{\max} of blend is lower than original one, might be interpreted as a result of interaction between SF and PU under thermal degradation condition. That is, SF and PU facilitate thermal degradation of blend partner each other.

Blood compatibility

Blood compatibility is one of essential properties in the application of biomedical fields. In general, blood coagulation is retarded by blood compatible material resulting in small amount of thrombus while it is accelerated by material with poor blood compatibility. Accordingly, it can be said that the amount of thrombus is intimately related to blood compatibility. Therefore, blood compatibility of SF/PU blend films was evaluated by the amount of thrombus produced. As appeared in Fig. 8, PU film shows 64% of adherent thrombus compared to that of untreated glass, showing a relatively good blood compatibility. The amount of thrombus was decreased with an increase of SF content in blends and only 29% of adherent thrombus was obtained for 70/30 SF/PU blend film.

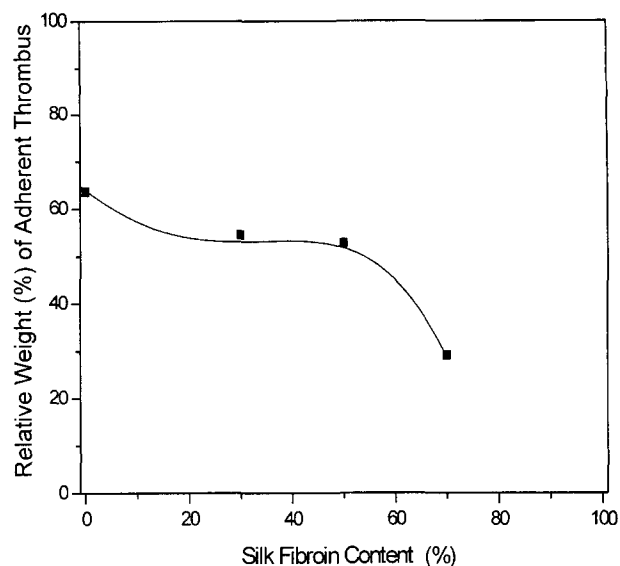


Fig. 8. Relative weight (%) of adherent thrombus for SF/PU blend films with various SF content.

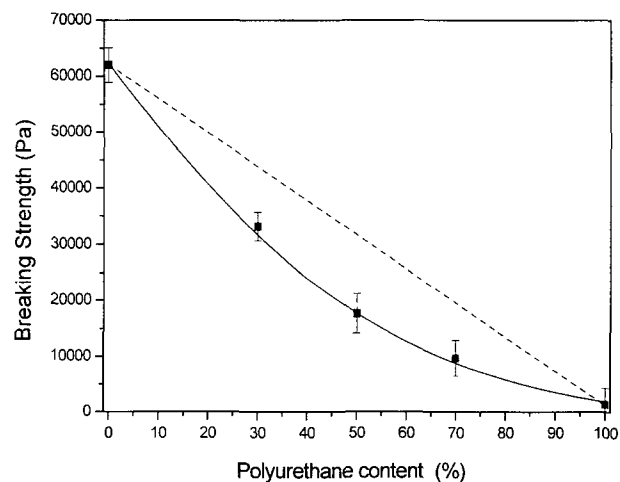


Fig. 9. Breaking strength of SF/PU blend films with various PU content.

Although blood compatibility of SF could not be evaluated here due to its solubility during the experiment, it is said that blood compatibility of PU can be improved by blending with SF polymer.

Mechanical property

Fig. 9 and 10 show breaking stress and strain of SF/PU blend films. SF and PU film shows 62 KPa and 1 KPa of breaking stress, respectively. SF/PU blend films exhibit lower breaking stress than the value expected from mixture rule. In general, mechanical stress is known to be intimately related to miscibility of blend. When polymer blend is miscible, breaking stress of blend deviate posi-

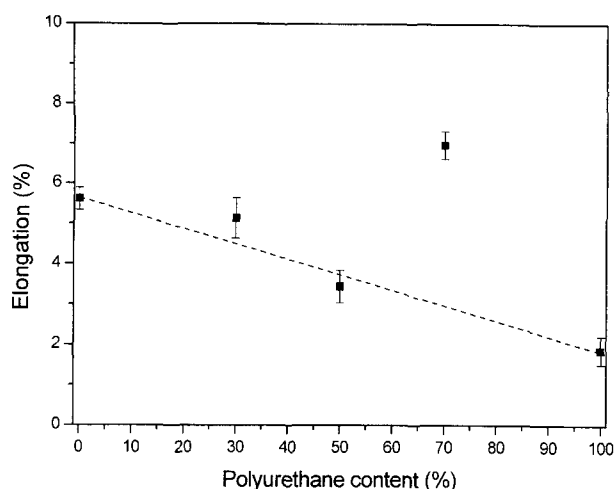


Fig. 10. Breaking elongation of SF/PU blend films with various PU content.

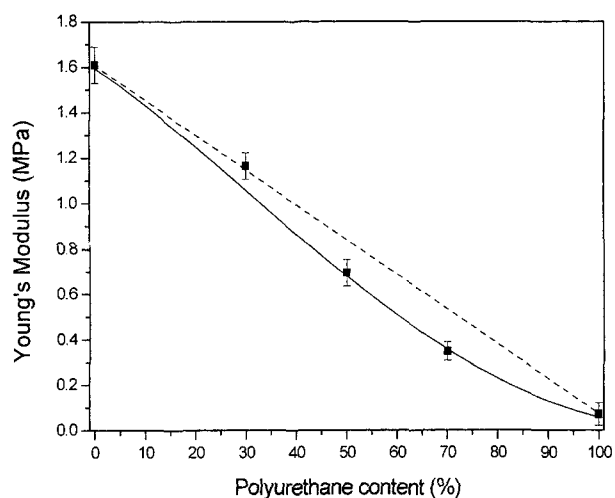


Fig. 11. Initial modulus of SF/PU blend films with various PU content.

tively from the value calculated from mixture rule. However, immiscibility results in a negative deviation showing poor mechanical properties. Therefore, a negative deviation of breaking stress for SF/PU blend films is due to the immiscibility and absence of specific interaction between blend components.

For breaking elongation (Fig. 10), SF film shows 5.6% and it decreases linearly with an increase of PU content. However, SF/PU blend with 70% PU content exhibits a positive deviation from the linearity. This might be related to miscibility of SF/PU blends. That is, higher degree of miscibility produces higher elongation. As seen in SEM observation, SF/PU blend with 70% PU displays higher miscibility than other blends. Therefore, SF/PU blend with 70% PU results in positive deviation from the linearity.

Initial Young's modulus reflects the brittleness of polymer. When a polymer has high Young's modulus, it is classified into brittle material. On the other hand, in opposite case, ductile one. The brittleness was evaluated from Young's modulus in this study and the results are presented in Fig. 11. SF film shows 1.6 MPa of initial Young's modulus. However, it decreases linearly with an increase of PU composition, indicating that the brittleness of SF film can be improved by mixing with PU.

PU polymer is known to have excellent mechanical properties. Therefore, it is expected that breaking strength and strain of SF is enhanced by the addition of PU in blend. However, in this study, adverse results were obtained due to the poor mechanical properties of PU polymer in blend. It seems because formic acid was used for dissolving and mixing of PU as a cosolvent, resulting from the deterioration of this polymer in this solvent. Nevertheless, blood compatibility of PU could be improved by blending with SF. Therefore, if a proper cosolvent is developed for SF and PU polymer, SF/PU blend will be a promising biomaterial for biomedical application fields due to an excellent blood compatibility.

Acknowledgements

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