## Swelling Behaviours of Silk Fibroin-Polyurethane Composite Hydrogels†

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Silk fibroin-polyurethane (SF-PU) hydrogels with different SF/PU ratios were fabricated. FTIR was applied to characterize the structure of the hydrogels. It was found that cross-linked network formed through chemical reactions between -NCO groups on the PU chain and -NH₂, -OH groups on the SF chains, in addition to the hydrogen bonding interactions. SEM was taken to observe the morphology of hydrogels exhibited a porous structure. The swelling ratio of hydrogel could be as high as 4.37 and it decreased with the increase of SF contents. Due to that the diffusion exponent (n) was about 0.45, the initial swelling stages were thought to be controlled by the Fickian diffusion. For the whole swelling process, experimental results well fitted into the Schott second-order kinetic equation.

Keywords: Silk fibroin-polyurethane hydrogels, Swelling behaviour, Fickian diffusion, Kinetic.

### INTRODUCTION

Hydrogels are generally water swollen polymer networks that formed by chemical/physical cross-linking. They can absorb large quantities of water without being dissolved[1]. Excellent hydrophilicity and biocompatibility have made hydrogels fast developed materials for various biomedical applications, such as Nucleus Pulposus replacement or the drug delivery systems[2,3].

As one of the common natural fibrous proteins, silk fibroin (SF) consists of 18 kind of α-amino acids. It has attracted significant attention as a biomedical material for the peculiar secondary structure, attractive biocompatibility and excellent thermostability[4]. However, SF hydrogels are limited to application because of weak mechanical properties[5]. Hence, concepts of blending SF with other polymers are put forward. Nowadays, various hybrid hydrogels have been fabricated, such as the SF/PNIPAAm, the fibroin/collagen and the SF/polyacrylamide, *etc.*[6,7].

Polyurethane (PU), composed of alternately linked soft / hard segments, has been widely studied for its outstanding properties. Polyurethane can be used as the biomedical materials for its biocompatibility, non-toxicity and long-term stability *in vivo*[8]. Based on these advantages, more and more PU-based hydrogels have been explored to utilize in many fields, such as tissue engineering, contact lenses, cardiovascular implants and drug release devices[9].

Up to now, hydrogels based on SF or PU has been extensively studied, while few hydrogel combining advantages of the two components together has been reported[10,11]. In the reports, SF-PU composite hydrogels were proven to be suitable for nanoparticle replacement. And swelling/deswelling behaviours of SF-PU hydrogels were studied. However, those detailed properties of hydrogels as well as the relationship between the performance and composition have not been expounded yet. Therefore, in present paper, hydrogels with different SF/PU ratio are prepared and investigated. The structure and morphology of the SF-PU hydrogels are characterized with FTIR and SEM. Swelling behaviours including swelling kinetics models are fully discussed. The results indicate that the SF/PU ratio affect the swelling behaviours of the SF-PU hydrogels.

### EXPERIMENTAL

Polyethylene glycol (PEG, Mn = 2000), isophorone diisocyanate (IPDI), polypropylene glycol (PPG, Mn = 2000) and dimethylol propionic acid (DMPA) were obtained from commerce. Dialysis tube (MWCO:8000-14000) was product of BIOSHARP. The cocoon of *B. mori* silkworm was supplied by Anhui Agricultural University.

Cocoons of *B. mori* silkworm were degummed 3 times in 0.5 % (w/v) $Na_2CO_3$ solution at 98-100 ºC for 0.5 h and then rinsed thoroughly with distilled water to extract the sericin proteins or wax. After dried, silk fibroins were dissolved in LiBr solution (9.5 mol/L), followed by a dialysis against distilled water to remove the salts and small molecules. The silk fibroin was further purified by centrifuge to get the final pellucid SF solution. Concentration of SF solution was 5.0 % (w/v), determined by the gravimetric method.

0.03 M PEG and PPG, 0.12 M IPDI were mixed into a round-bottom four-necked flask equipped with a mechanical stirrer, a reflux condenser, a nitrogen inlet and a thermometer. After being stirred at 85 ºC for 2 h, the reactor was cooled to 60 ºC. Next, the mixture was extended by the addition of 0.025 M DMPA and catalyst. The system maintained at 70 ºC for 5 h, while acetone was added to decrease viscosity. After cooled to 40 ºC, mixture was neutralized by 0.025 M triethylamine (TEA), then got the polyurethane pre-polymer.

Silk fibroin solution and polyurethane were mixed with quick stirring at room temperature and poured in cylindrical moulds, forming the SF-PU hydrogels. After the gelation completed, gels were cut into pieces 8 mm long and then vacuum-dried at 40 ºC for 3 d. Gels with different compositions (Table-1) were synthesized by adjusting the ratios of SF/PU.

FTIR spectra were recorded using a spectrometer (Nicolet NEXUS-870, USA) in the range of 4000-400 $cm^{-1}$. Samples for SEM measurement first swelled in distilled water, thereafter the swollen samples were dehydrated in absolute ethanol and then dried in vacuum at 30 ºC for 1 d[12]. After being sputtered with gold, cross-sectional morphologies of hydorgels were tested at an accelerating voltage of 5.0 kV by SEM (S-4800, Japan). A series of pre-weighed dried samples were first immersed in distilled water. At regular time intervals, swollen hydrogels were removed from the water and weighted after the excess surface liquids were wiped. Swelling ratio (SR), evaluating the swelling properties of hydrogels, was defined as:

$$SR = \left[ \frac{(W_t - W_0)}{W_0} \right] \qquad (1)$$

where $W_t$, $W_0$ represented the weight at time t and the initial weight of the hydrogels, respectively.

## RESULTS AND DISCUSSION

FTIR spectra of hydrogel (SF-PU2), PU and SF were shown in Fig. 1. Infrared spectra (b) showed the typical structures of PU: the peaks at around 3350 $cm^{-1}$ was related to the N-H stretching vibration; the typical peak at 2265 $cm^{-1}$ are related to -NCO groupsthe partly H-bonded carbonyl stretching peaks can be observed at 1718 $cm^{-1}$; the strong absorption at 1109
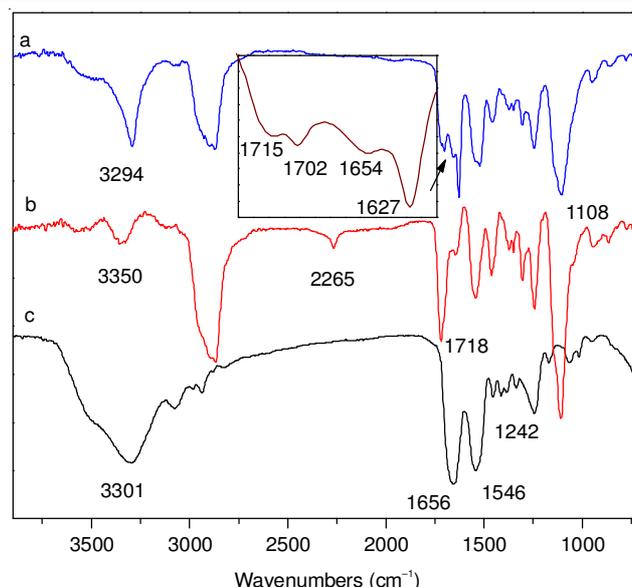


Fig. 1. FTIR spectra of (a) SF-PU2 hydrogel (b) PU and (c) SF

$cm^{-1}$ was assigned to the stretching vibration of the ether groups. Curve (c) indicated the characteristic spectrum of SF in random structure: the broad band at 3301, 1656, 1546 and 1242 $cm^{-1}$ were assigned to the N-H stretching vibration, amide-I (C=O), amide-II (N-H) and amide-III (C-N), respectively. Compared with PU, hydrogel spectrum showed the part of the characteristic PU bands [Fig. 1(a)]. After SF was added, the corresponding N-H bands shifted from 3350 to 3294 $cm^{-1}$ and became stronger. The carbonyl band notably weakened and shifted to 1715 $cm^{-1}$, while peak at 1702 $cm^{-1}$ as shoulder became stronger relatively. It indicated that more H-bonding was formed between amide groups in hydrogel. Besides, the sharp peak observed at 1627 $cm^{-1}$ was attributed to the carbonyl of the urea groups. It was product of $-NH_2$ of SF and -NCO group of PU, which can be seen as the chemical cross-linking points of hydrogel. In spite of partly overlapped with PU bands, the characteristic bands of SF can be traced in hydrogel spectrum. Hence, it is assumed that SF chains are physically and chemically cross-linked with PU chains, forming the ideal network structure [11].

SEM image of dried gel (SF-PU2) was presented in Fig. 2. The sample exhibited irregular porous structure. Diameters of pores were in the range of 50-300 mm and those pores may enlarge the surface area of the network. Due to the hydrophilic of the SF and PU chains, water molecules can penetrate through the hydrogels easily and retain in such pores. Besides, the compact backbone of hydrogels showed in image can largely contribute to the mechanical properties.

To investigate the swelling behaviour of gels and the impact of SF contents, samples with different SF/PU ratios were

| | | | Fickian diffusion | | | Schotts kinetics | | | $D \times 10^5$ |
|---|---|---|---|---|---|---|---|---|---|
| Samples | SF/PU (w/w) | ESR | n | K | R | $S_\infty$ | $k \times 10^2$ ($h^{-1}$) | R | ($cm^2$/min) |
| SF-PU1 | 1/30 | 4.37 | 0.45 | 0.18 | 0.9992 | 4.67 | 2.54 | 0.9998 | 7.91 |
| SF-PU2 | 1/25 | 4.21 | 0.44 | 0.19 | 0.9997 | 4.41 | 2.24 | 0.9995 | 7.21 |
| SF-PU3 | 1/20 | 3.97 | 0.43 | 0.20 | 0.9993 | 4.22 | 2.78 | 0.9997 | 7.82 |
| SF-PU4 | 1/15 | 3.85 | 0.43 | 0.20 | 0.9983 | 4.11 | 2.56 | 0.9996 | 7.34 |

TABLE-1
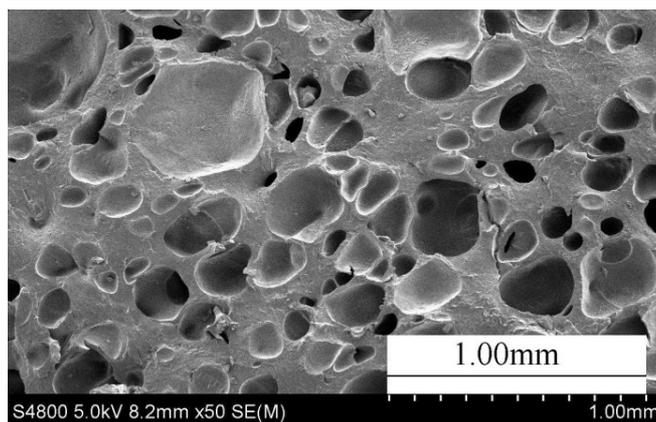PROPORTIONS AND SWELLING KINETIC PARAMETERS FOR SF-PU HYDROGELS

Fig. 2. SEM image of SF-PU2

measured in distilled water at 15 ºC and the swelling ratio-time curves were recorded in Fig. 3. It was observed that the swelling ratio of gels increased rapidly in the first 24 h and then became slower till reaching equilibrium. As the SF/PU ratios of gel increased, the corresponding equilibrium swelling ratios (ESR) decreased, indicating the swelling behaviour was dependent on SF content. In these gels, PEG segments were hydrophilic and can absorb large amount of water. The numerous, large and interconnected pores in gels also played critical role in leading to a fast swelling rate and retained water. However, for this system, a higher SF content suggested a higher chemical cross-linking density, which may restrict polymer relaxation and go against the swelling process[13]. Considering that PEG in the gels was almost constant, the fact that the ESR values declined with SF content can be attributed to the increment of chemical cross-linking points.
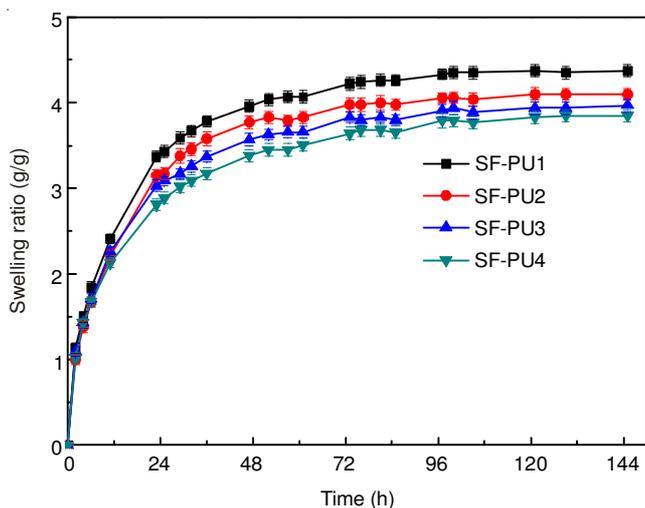


Fig. 3. Effect of different SF/PU ratio on the swelling kinetics of hydrogels

To better elucidate the controlling mechanisms of SF-PU gel's swelling process, two classical kinetic theories were applied, one was Fickian diffusion model and the other was Schotts second-order kinetic equation.

For Fickian diffusion, three steps occurring in succession are proposed when a dry gel swells in water *i.e.*, first, water molecules diffuse into the polymer network; second, the hydrated polymer chains relax and third, the polymer network expands

into the surrounding. At the initial swelling stage ($M_t/M_\infty \leq$ 60 %), following equation was used to describe the penetrating mechanism:

$$F = \frac{M_t}{M_\infty} = Kt^n \qquad (2)$$

where $M_t$ and $M_\infty$ was the mass of water absorbed at time t and at equilibrium, respectively; K was a characteristic constant related to network structure of hydrogels and n was the diffusion exponent. For n < 0.5, Fickian diffusion would be dominant; for 0.5 < n < 1, the transport would be anomalous (non-Fickian diffusion) and for n = 1, the relaxation of polymer chains would control the system[14]. Plots of ln F *versus* ln t of different gels were shown in Fig. 4. n and K calculated from slopes and intercepts of the lines were listed in Table-1. It was clear that the results exhibited excellent linear correlations and n values were all in the range 0.43-0.45, indicating the swelling behaviour was dominated by Fickian diffusion at the initial swelling stage. Both the values n and K of gels were close to each other.
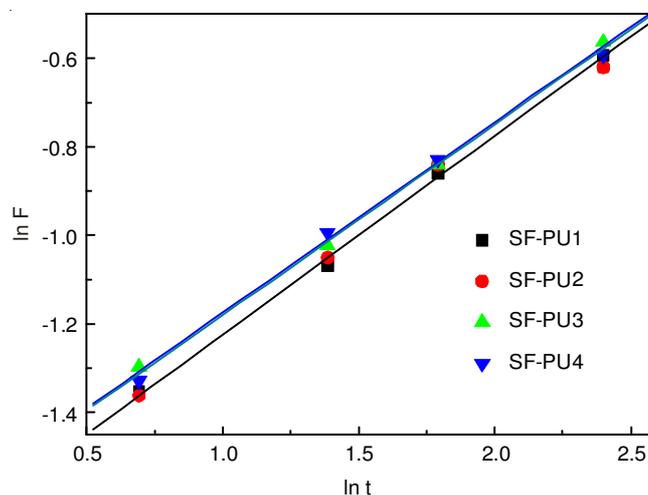


Fig. 4. ln t dependence of ln F for different hydrogels

As to the whole swelling process of gels, the Schotts second-order kinetic equation was of great significance in testing the experimental data[15] and the swelling kinetic analysis was described in form:

$$\frac{dS}{dt} = k(S_\infty - S)^2 \qquad (3)$$

After definite integration yields: t/S = A + Bt, where k was the rate constant, S and $S_\infty$ denoted the swelling ratio at time t and theoretical equilibriums state, respectively. A = 1/k × $S_\infty^2$ was the reciprocal of initial swelling rate and B = $1/S_\infty$ was inverse of the theoretical equilibrium swelling ratio. Through t/S *vs.* t in Fig. 5 the kinetic parameters were calculated from the slopes and intercepts of the lines and listed in Table-1. The related coefficients (R) were all beyond 0.999, suggesting that the Schotts second-order kinetic equation was suitable in describing the whole swelling process. The values of $S_\infty$ were slightly larger than the experimental ones and also increased with the decrease of SF content. In addition, the rate constant k values were different from each other, suggesting different swelling rates of hydrogels.
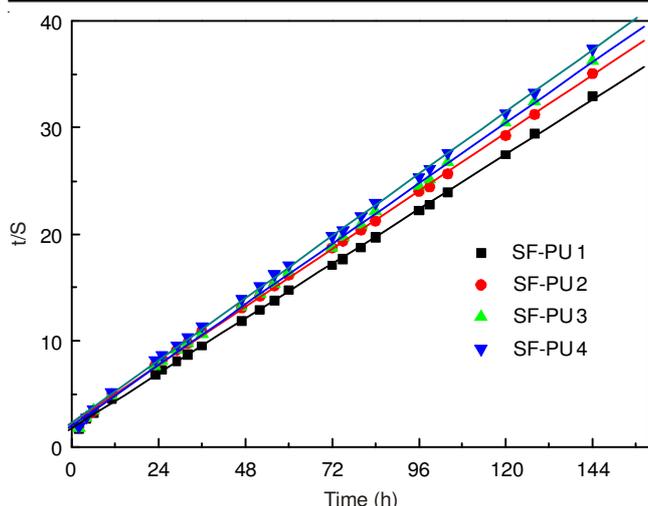
Fig. 5. Relationships of t/S with t for different hydrogels

For the characterization of the hydrogels, the diffusion coefficient (D) can be determined by different methods. The short-time approximation method is used for the calculation of D. this method is used for the first 60 % of swelling of cross-linked polymers in a chosen solvent. Commonly, D of cylindrical hydrogel is determined by the following equation[16]:

$$F = \frac{M_t}{M_\infty} = 4\left[\frac{Dt}{\pi r^2}\right]^{1/2} - \pi\left[\frac{Dt}{\pi r^2}\right] - \left(\frac{\pi}{3}\right)\left[\frac{Dt}{\pi r^2}\right]^{3/2} \quad (4)$$

where D ($cm^2$/min), t is the time (min) and r is the radius (cm) of cylindrical samples. D of the samples was calculated from the slope of the lines of F against $t^{1/2}$. The results are listed in Table-1. It can be seen that the D values keep the constant with the SF/PU increase.

**Conclusion**

Composite hydrogels based on silk fibroin and polyurethane were prepared. Increase in silk fibroin content of hydrogels resulted in a higher chemical cross-linking density in samples. The increased chemical cross-linking density may restrict the relaxation of molecular chains and go against the swelling process. The swelling ratios decreased with the increase of cross-linking density, indicating that compared to physical interaction, chemical cross-linking played the main role in these hydrogels.

At the initial swelling stage, it was thought that the Fickian diffusion dominated in hydrogels. For the whole swelling process, the experimental results could be well fitted into the Schott second-order kinetic equation. Moreover, the diffusion coefficients of the samples kept constant with the change of SF/PU ratios.

According to the results of SEM, the samples have similar porous structure. At the initial swelling stage, water molecules entered into the gels through the Fickian diffusion. So all the hydrogel samples have about the same swelling behaviours. The diffusion coefficient, the diffusion exponent n and the characteristic constant related to network structure of hydrogels K kept almost constant for all samples. But when the SF/PU ratio increased the ESR or $S_\infty$ decreased obviously.

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