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New Oral Dosage Form for Elderly Patients: Preparation and Characterization of Silk Fibroin Gel

Takehisa HANAWA,* Atsushi WATANABE, Takanori TSUCHIYA, Rieko IKOMA, Masato HIDAKA, and Masayasu SUGIHARA

Pharmaceutical Department, Tokyo Women's Medical College, Kawada-cho, Shinjuku-ku, Tokyo 162, Japan.

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The pharmaceutical utility of silk fibroin as a possible material for an oral dosage form for elderly patients was investigated. Silk fibroin gel (SFG) was prepared from its aqueous solution. The gel formation was studied as a function of adjusted pH and concentration of silk fibroin (SF). On the basis of Fourier transform infrared spectroscopy of SFG, the transition from the random coil to the β-structure was observed. The rate of gelation was sufficiently accelerated by the addition of glycerol to the SF aqueous solution. The glycerol content also affected the rate of gelation of the SF solution. Rheological properties of SFG were evaluated using a creep meter. The SF content and/or glycerol content affected the breaking stress of SFG. Moisture desorption from SFG was retarded with an increase in glycerol content. It was found that SFG was able to be prepared at room temperature (20±5 °C), and the SF content and glycerol content affected the formation and physicochemical properties of SFG.

Key words silk fibroin; gel; gelation time; breaking stress; water vapor sorption

It has been reported that jelly-like preparations are available as an oral dosage form for the elderly[1] because of their easy handling and swallowing. These jelly-like preparations have been prepared with various materials, such as sodium caseinate,[2] glycerogelatin[3] and dried gelatin gel powder.[4] In these materials, however, a heating procedure is required to prepare the gel form. Therefore, the thermolabile compounds are limited in their use as active constituents.

Recently, silk fibroin (SF) has been used in various research fields such as the medical,[5] biomaterial,[6] and food additive[7] fields because of its unique physicochemical properties and its harmlessness to humans. SF forms the principal constituent of the fibrous protein of *Bombyx moli.*, and is one of the scleroproteins. Chuan *et al.* demonstrated that SF can be stabilized in the β-structure by intermolecular hydrogen bonds between polypeptide chains.[8]

In this study, we have attempted to prepare silk fibroin gel (SFG) as a new oral dosage form for the elderly. The physicochemical properties of SFG were investigated by means of Fourier transform infrared spectroscopy (FTIR), rheological measurement, and water vapor sorption measurement.

Experimental

Materials Silk thread was purchased from Kayamachi, Kyoto, Japan. Calcium chloride was of reagent grade. Glycerol for serovaccine of 98.5% purity was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Preparation of SF Powder Silk thread was refined according to the procedure of Tsukada *et al.*[9]: 20 g of silk thread (Fig. 1a) was washed with distilled water and purified in 0.5% $NaHCO_3$ solution at 100 °C for 30 min. The purified silk thread was dissolved in 50% (w/v) calcium chloride solution at 100 °C and dialyzed using a cellulose tube for 3 d under a distilled water stream. The solution was lyophilized with a freeze drier (EYLA FD81, Tokyo, Japan), and SF powder was obtained (Fig. 1b, Chart 1).

Preparation of SFG Various amounts of SF powder (0.2—4.0 g) were dissolved in 20 ml of distilled water[7] or in glycerol solution (10—50% (v/v)) at room temperature (20±5 °C), and the pH of the mixture was adjusted to 3.00 or 4.00 with 1 M citric acid. The mixture was transferred to a 2.5 ml container and stored at 4 °C (Chart 2).

Determination of Gelation Time Gelation time, the time required for the formation of a gel from solution, was determined according to the

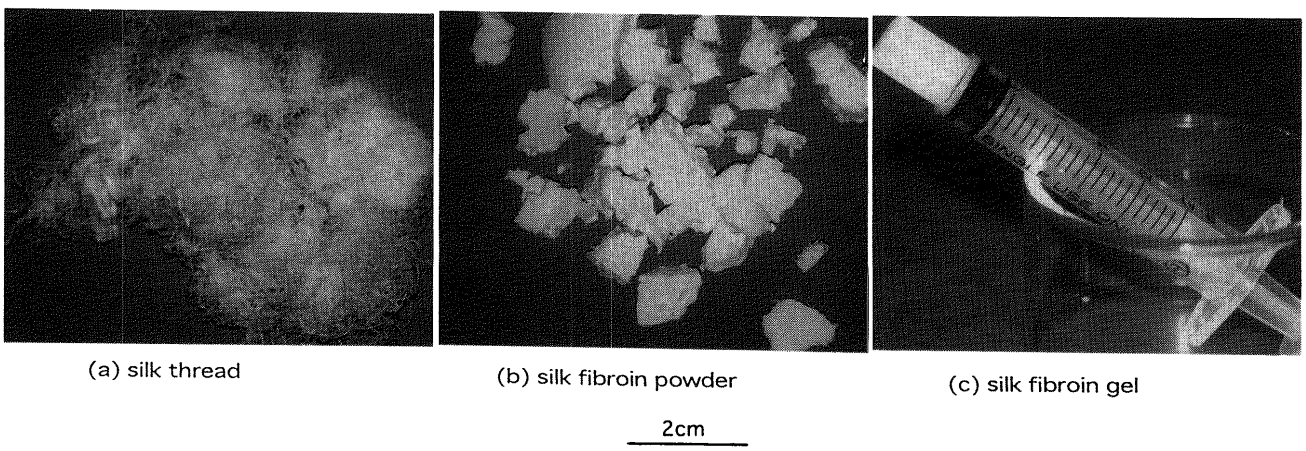

Fig. 1. Photographs of Silk Fibroin Gel

a, silk thread; b, silk fibroin powder; c, 10% silk fibroin gel (silk fibroin: glycerol: water = 10 : 45 : 45).

* To whom correspondence should be addressed.

preparation of fibroin powder
2.0 g of silk thread
↓
dissolved in 100 ml of 50% (w/v) calcium chloride solution at 100°C
↓
dialysis for 3 d in water stream
↓
lyophilization
↓
fibroin powder

Chart 1

preparation of silk fibroin gel
(0.2—4.0 g) of silk fibroin powder
↓
dissolved in 20 ml of (0—50% (v/v)) glycerol solution
↓
pH adjustment (pH 3.00 or 4.00) with 1 M citric acid
↓
transference of the mixture into the 2.5 ml container
↓
silk fibroin gel
↓
stored at 4°C

Chart 2

procedure of Oakenfull *et al.*[10] A fixed weight (5.0 g) of solution was placed in a 10 ml flat-bottomed cylindrical vial (diameter: 21 mm). The vial was tilted at definite time intervals, and the time required to form a gel just strong enough to retain its shape in position was recorded.

Rheological Measurement Rheological measurements were carried out using a creep meter (Yamaden model 33005, Tokyo, Japan), that is, cubically cut SFG (2 cm × 2 cm × 2 cm) was in contact with a plunger, and the thickness of the SFG was measured. SFG was loaded at the rate of 1 mm/s, and the stress force and strain were measured at the moment the SFG broke.

Water Vapor Sorption Study of SFG A known weight (1.0 g) of SFG was stored in a desiccator for humidification at 20 °C. Phosphorus pentoxide and aqueous saturated salt solution were used to get specific relative humidities (RHs). The initial water content of SFGs prepared in 10, 25 and 50% glycerol solution was 85.5, 71.3 and 49.5%, respectively. The extent of water vapor sorption was periodically monitored by measuring the weight of the samples with an electrical balance (Shimadzu Libror AEG-120, Kyoto, Japan).

Infrared (IR) Absorption Spectroscopy The measurements were carried out on a Jasco VALOR-III Fourier transform IR spectrophotometer (Tokyo, Japan) using the attenuated total reflection method. Germanium crystal was used as an attenuated total reflection (ATR) crystal. SF aqueous solution or SFG was put in and stuck to the ATR crystal plates.

Melting Point Measurement of SFG The melting point measurement of SFG was carried out according to the method of Watanabe *et al.*[3] A teflon bar (length: 8 mm) was put in the bottom of a screw cap test tube, and the SF solution was transferred. After gelation, the test tube was allowed to stand upside down in a water bath and was heated at a rate of 0.5 °C/min. The melting point was defined as the temperature at which the teflon bar fell from the bottom of the screw cap test tube during heating.

Disintegration Test of SFG The disintegration test of SFG was performed according to JP XII. Distilled water was used as the test fluid.

Results and Discussion

Gelation of SF Aqueous Solution Figure 1c is a photograph of the SFG in a disposable syringe. The SFG was white-colored, tasteless and odorless. Shimura[11] demonstrated that the gelation of SF took place while its acidic solution stood, and was due to the transition from the random coil to the β-structure by intermolecular hydrogen bonds between its polypeptide chains. The gelation times with various SF content and pH conditions are listed in Table I. When the pH of the mixtures was adjusted to 2.00, gelation was not observed, whereas in the case of pH 3.00 and 4.00, gelation was observed. The gelation time decreased with an increase in fibroin content. At pH 4.86, which is the isoelectric point of fibroin aqueous solution, gelation was not observed. This was explained by presuming the existence of an optimal pH for gelation. From the melting point measurement, SFG did not indicate a definite melting point. In the case of jelly or gel forms, since gelatinization gives rise to thermal reversibility, thermal stability throughout storage should be considered. In contrast, SFG is thermally irreversible, and this seems to be favorable under various storage conditions.

TABLE I. Gelation Time of Silk Fibroin Gel as a Function of Silk Fibroin Content or Adjusted pH

Silk fibroin content (%)	pH			
	2.00	3.00	4.00	4.86
1.0	NR	5 d	10 d	NR
2.0	NR	5 d	5 d	NR
5.0	NR	2 d	2 d	NR

NR, gelation was not recognized.

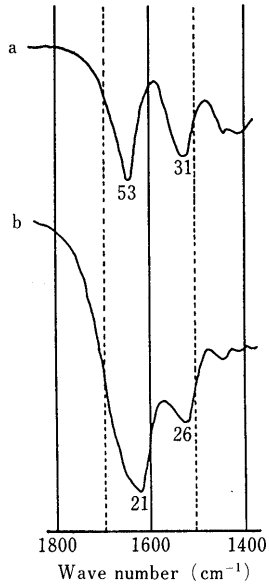

Fig. 2. Infrared Spectra of Silk Fibroin and Silk Fibroin Gel
a, silk fibroin aqueous solution; b, silk fibroin gel.

Figure 2 shows the IR spectra of the SF aqueous solution and SFG. In the spectrum of the SF aqueous solution, the amide I and amide II bands were observed at 1653 and 1531 cm^{-1}, respectively. Iizuka *et al.* demonstrated that SF in aqueous solution at neutral pH had a random coil conformation.[12] On the other hand, in the spectrum of SFG, these peaks were shifted to a lower frequency at 1621 and 1526 cm^{-1}, respectively, indicating the transition from the random coil to β-structure during gelation of the protein.[13] In practical use, however, this experimental system is too time-consuming to form the SFG, and because of the presence of a significant amount of water

in the SFG, from a chemical and microbiological point of view, it seems to be unfavorable to use SFG as an oral dosage form.

Gelation of SF Glycerol Solution Oakenfull *et al.* demonstrated that the gelation of gelatin gel was accelerated by the addition of sugar and polyols.[10] In order to shorten the time required for the gelation of SF, we used the glycerol solution as a solvent. Figure 3 shows the gelation time of 5% SFG as a function of glycerol content, where the time required for gelation is designated in parentheses. The gelation time sufficiently decreased with an increase in glycerol content. Magoshi demonstrated that the β-transition of the SF (random coil) solution took place by adding methyl alcohol (MeOH), and the transition time decreased with an increase in MeOH concentration.[14] Furthermore, it was stipulated that organic solvent contributing to the β-transition was polar, hydrophilic, and contained a dehydrative solvent such as MeOH, ethyl alcohol or *n*-propyl alcohol. Magoshi also assumed the mechanism of β-transition to act in the following manner: (1) The SF (random coil) is swelled by the water molecule contained in the MeOH solution, and the hydration of SF proceeds simultaneously. (2) The MeOH molecule diffuses into and dehydrates the SF (random coil) molecule. (3) Sharing of the SF (random coil) takes place during hydration, and the β-transition may result.[14] On the other hand, Gekko *et al.* demonstrated that the addition of glycerol to proteins caused the spherical exclusion of glycerol from the protein domain and also caused preferential hydration and stabilization of the protein.[15] That is to say, since glycerol interacts favorably with water, the presence of glycerol in the aqueous medium could increase the hydrophobicity of the protein.[13] From these considerations, in the case of the SFG prepared with a high glycerol content, polypeptide chains may be brought very close to form the β-structure in a short time. Figure 4 shows the gelation time of SFG as a function of the SF content. The gelation time decreased with an increase in SF content. This apparently indicates that the portion of the polypeptide chain which forms the β-structure increased. To summarize this study, the gelation time of SFG depended upon the glycerol content and the SF content.

Rheological Study of SFG Yanagisawa *et al.* demonstrated that the physical properties of an oral dosage form, such as hardness and shape of the material, affected the number of chewing strokes.[16] For example, mastication of gummy materials requires a number of chewing actions but no swallowing action. Elderly patients have much difficulty taking certain dosage forms because of impaired swallowing.[3] From this point of view, it seems that a suitable fragility is necessary for jelly-like preparations to provide easier swallowing. Furthermore, it has been reported that jelly- or pudding-like preparations are available as an oral dosage form for the elderly[1] because of their easy handling and swallowing compared to syrup or powders, and/or their good texture, similar to commercial jelly or pudding. To evaluate the texture of various food hydrocolloids, rheological measurements have been carried out. In the case of SFG, since disintegration was not observed during 60 min of measurement, chewing action is required for disintegrating the SFG. To evaluate the texture of the SFG, rheological measurement was carried out. Figure 5 illustrates the stress-strain curves of SFG with various SF content. In the stress-strain curve, the slope of the initial portion indicates the initial modulus of elasticity of SFG; the smaller the slope of the initial portion, the more facile the SFG deforms. That is, SFG which shows a small breaking point and slope has suitable fragility. In this study, the breaking point of each gel, which indicate the firmness of SFG and the rupture of the SFG taking place at this point, is shown by arrows. In each stress-strain curve, since the yield points coincided with the breaking point, these behaviors are confirmed as a brittle fracture. The load at the breaking point increased with an increase in SF content. This was explained by presuming the existence of a closely networked SFG at the higher SF concentrations. On the other hand, with the addition of glycerol, SFGs showed lower breaking points. In this study, the reason for the decrease in breaking point by increasing the glycerol content is not yet fully understood, but it might be related to the increase in viscosity among the

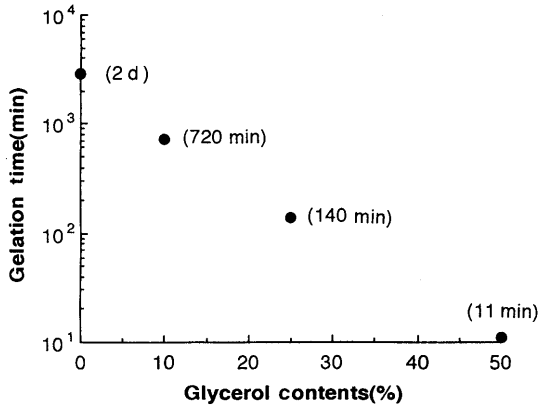

Fig. 3. Gelation Time of 5% Silk Fibroin Gel as a Function of Glycerol Content

pH was adjusted to 4.00

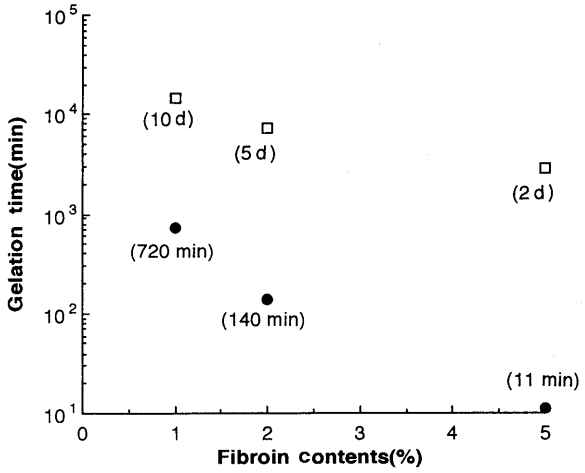

Fig. 4. Gelation Time of Silk Fibroin Gel as a Function of Silk Fibroin Content

pH was adjusted to 4.00. ●, prepared in 50% glycerol solution; □, prepared in distilled water.

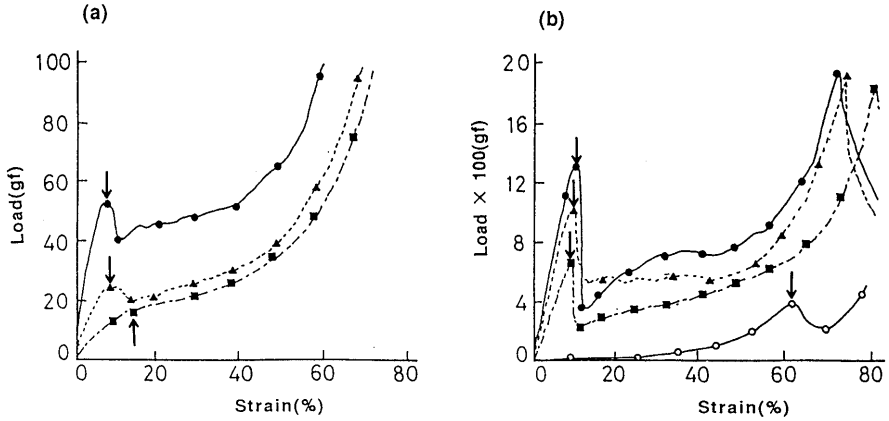

Fig. 5. Stress-Strain Curves of Silk Fibroin Gel

a: 2% silk fibroin gel. ■, prepared in 50% glycerol solution; ▲, prepared in 25% glycerol solution; ●, prepared in 10% glycerol solution.
b: 5% silk fibroin gel. ■, prepared in 50% glycerol solution; ▲, prepared in 25% glycerol solution; ●, prepared in 10% glycerol solution; ○, 5% gelatin gel.

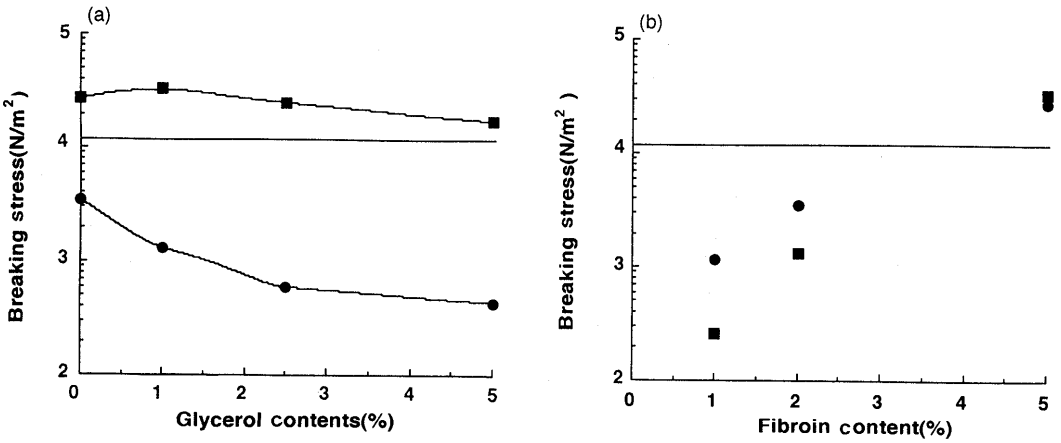

Fig. 6. Breaking Stress of Silk Fibroin Gel as a Function of Glycerol Content or Silk Fibroin Content

a: breaking stress of silk fibroin gel with various glycerol content. ■, 5% silk fibroin gel; ●, 2% silk fibroin gel.
b: breaking stress of silk fibroin gel with various silk fibroin content. ■, prepared in 10% glycerol solution; ●, prepared in distilled water. Horizontal line shows the breaking stress of 5% gelatin jelly.

SFG. The gel breaking strain indicates the fragility of the material. In the 2.0 and 5.0% SFGs, the gel breaking strains were as low as 8.60 and 10.7%, respectively. These values were about one-sixth lower than that of the 5% gelatin jelly normally used for commercial jelly. This indicates that SFGs are more rigid and brittle, meaning that SFGs have both suitable fragility for easier swallowing and facility in disintegrating in the mouth or the esophagus. Figure 6a shows the breaking stress of SFG as a function of the glycerol content. The breaking stress of 5% SFGs was 7.9—39.6 times greater than that of 2% SFGs. Though the role of glycerol in determining the breaking stress of SFG could not be revealed in the present study, it appears that added glycerol enhances the intermolecular forces involved in forming SFG, and an excess amount of glycerol, which does not contribute to gel formation, leads to a decrease in elasticity. Figure 6b shows the breaking stress of SFG as a function of the SF content. The breaking stress of SFG increased with an increase in SF content. However, since the 1.0 and 2.0% SFG were shaped like rice porridge, it seemed to be unfavorable to handle.

From these results, it was found that 5% SFG showed a suitable fragility and breaking stress, similar to 5% gelatin jelly, meaning that the same texture as 5% gelatin jelly is expected.

Water Vapor Sorption Study of SFG Watanabe *et al.* demonstrated that water vapor desorption was observed on glycerogelatin during storage under various relative humidities (RHs), and a device for packaging is required for practical use.[3] In this study, to evaluate the methods of preserving SFGs, the water vapor sorption behavior of SFGs was investigated. Figure 7 illustrates the water vapor sorptions pattern of 5% SFG prepared at various glycerol contents and stored under various RHs at 20 °C. The points shown are the average values of three measurements. At 0% RH, water vapor sorption patterns showed similar moisture desorption, and almost all of the moisture was desorbed after 4 d of storage. Shrinkage was observed for each SFG. In the case of 58% RH, moisture desorption was depressed with an increase in glycerol content. In contrast, at 94% RH, moisture adsorption was observed in the SFG prepared in a 50% glycerol solution. It seems that glycerol is so hygroscopic that it absorbs the moisture during high RH storage.

In conclusion, SFG could be obtained at a room temperature (20±5 °C), and with the addition of glycerol

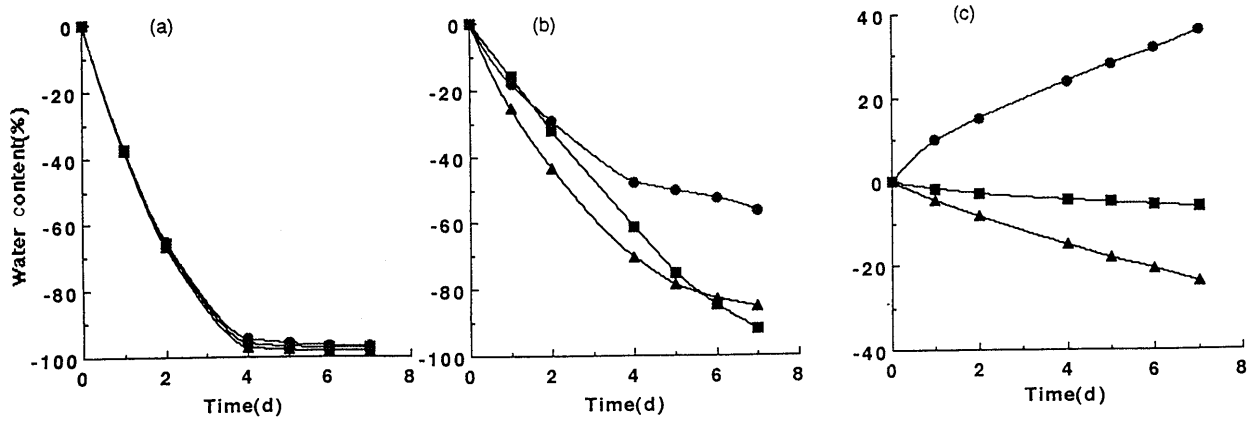

Fig. 7. Water Vapor Sorption Patterns of Silk Fibroin Gel Stored under Various Relative Humidities (RH) at 20°C

a, stored under RH 0%; b, stored under RH 58%; c, stored under RH 94%. ●, prepared in 50% glycerol solution; ■, prepared in 25% glycerol solution; ▲, prepared in 10% glycerol solution.

to SF aqueous solution, its gelation time was significantly shortened. The glycerol content affected the rate of gelation, shear modulus and water vapor sorption of SFG. For this reason, SF is able to exist in a powder form during storage, then it changes to a jelly-like form with the addition of glycerol solution. As regards the stability of the drug in the jelly, Watanabe *et al.* reported that the hydrolization of trichloromethiazide (TCM) is more regulated in glycerogelatin than gelatin jelly.[3] That is, the presence of glycerol decreases the number of water molecules which contribute to the hydrolysis of TCM. Therefore, in the case of SFGs, the same effect is to be expected regarding chemical and/or microbiological stability; it seems that SFG is superior to other jelly-like forms.

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References

1) M. Sugihara, *Medical Digest*, **39**, 51 (1990).
2) A. Watanabe, M. Sugihara, *Yakuzaigaku*, **52**, 69 (1992).
3) A. Watanabe, T. Hanawa, M. Sugihara, *Yakuzaigaku*, **54**, 77 (1994).
4) A. Ito, Y. Dobashi, K. Obata, M. Sugihara, *Byoin Yakugaku*, **20**, 41 (1994).
5) M. Iwatsuki, T. Hayashi, H. Funaki, *J. Adhesion Soc. Jpn.*, **27**, 410 (1991).
6) N. Minoura, M. Tsukada, M. Nagura, *Biomaterials*, **11**, 430 (1990).
7) K. Hirabayashi, Y. Hiraiwa, *New Food Industry*, **35**, 17 (1993).
8) C. X. Liang, K. Hirabayashi, *J. Applied Polym. Sci.*, **45**, 1937 (1992).
9) M. Tsukada, Y. Goto, N. Minoura, *J. Seric. Sci. Jpn.*, **59**, 325 (1990).
10) D. Oakenfull, A. Scott, *Food Hydrocolloids*, **1**, 163 (1986).
11) K. Shimura, E. Iizuka, "Tanpakushitsu No Kagaku," Vol. 4, Kyoritsu Shuppan Co. Ltd., Tokyo, 1973, pp. 711—763.
12) E. Iizuka, J. T. Yang, *Proc. Natl. Acad. U.S.A.*, 55, 1175 (1966).
13) S. Yu. Venyaminov, N. N. Kalmin, *Biopolymers*, **30**, 1259 (1990).
14) J. Magoshi, *Koubunshi Ronbunshu*, **31**, 765 (1974).
15) K. Gekko, S. N. Timascheff, *Biochemistry*, **20**, 4667 (1981).
16) K. Yanagisawa, K. Shiozawa, S. Yoshino, M. Matsuura, K. Seto, H. Sakanishi, T. Ohki, A. Habuto, *Tsurumi Univ. Dental J.*, **16**, 63 (1990).
17) A. Murayama, S. Osako, A. Kawabata, *J. Home Econ. Jpn.*, **41**, 133 (1990).