New Oral Dosage Form for Elderly Patients. II.[1) Release Behavior of Benfotiamine from Silk Fibroin Gel

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Silk fibroin gel (SFG) containing benfotiamine (BTMP) was prepared. The release behavior of BTMP from SFG was studied as a function of silk fibroin (SF) content and glycerol content, and the influence of the existence of β-cyclodextrin (β-CD) on the physicochemical properties of SFG were investigated. The release rate of BTMP from SFG was retarded by an increase in SF concentration. The addition of β-CD affected both the release properties and rheological properties of the SFG. It was found from the results of the "paddle-bead method" that the release profiles of BTMP from SFG were inversely proportional to the SFG firmness.

Key words silk fibroin gel; benfotiamine; β-cyclodextrin; release behavior; glycerol; paddle-bead method

Silk fibroin (SF) is used in various research fields such as those of cosmetics,[2)] medical materials,[3)] and food additives[4)] because of its unique physicochemical properties.

In a previous paper, we reported that silk fibroin gel (SFG) could be prepared from SF aqueous solution at room temperature (20±5 °C).[1)] The rate of gelation was dependent upon glycerol content and/or SF content, and addition of glycerol to the SF solution accelerated this rate. The amount of glycerol added and SF content affected the breaking stress of SFG.

In this study, we selected benfotiamine (BTMP) as the model drug and prepared SFG containing BTMP. BTMP is a vitamin B_1 derivative, and has been widely used for thiamine deficiency; however the drug is only slightly water soluble, as a result of which it may exhibit poor absorption characteristics. We therefore used β-cyclodextrin (β-CD) as a solubilizer of BTMP and investigated the release behavior of BTMP from SFG, and the influence of the presence of β-CD on the physicochemical properties of SFG.

Experimental

Materials Silk thread was purchased from Kayamachi, Kyoto, Japan. Glycerol for serovaccine (min: 98.5% purity) was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). BTMP was generously supplied by Sankyo Co., Ltd., (Tokyo, Japan). β-CD was purchased from Nacalai Tesque Inc.(Kyoto, Japan) and used without further purification.

Preparation of SFG Containing BTMP Various amounts of SF powder (0.2—4.0 g) were dissolved in 20 ml of distilled water or in glycerol solution (10—50 v/v%), and a fixed weight (0.15 g) of BTMP crystals passed through 100 mesh sieves was dispersed at room temperature (20±5 °C). The pH of the mixture prepared in this manner was then adjusted to 4.00 with 1 M citric acid, and the mixture was placed in a 2.5 ml cylindrical container (diameter: 9 mm) and stored at 4 °C.

Preparation of SFG with β-CD A mixture of β-CD (0.65 g) and BTMP (0.15 g) was dissolved in 20 ml of distilled water or 10—50% glycerol solution, and the same procedure followed as above.

Evaluation of BTMP Release from the SFG The release behavior of BTMP from SFG was investigated by the Japan Pharmacopoeia (JPXII) paddle method (paddle rotating speed: 100 rpm) at 37 °C. Distilled water was used as the test fluid. Various weights of cylindrical SFG (diameter: approx. 9 mm, length: approx. 12 mm) corresponding to 7.5 mg of BTMP were added to each test fluid. A 5.0 ml portion of the fluid was removed at suitable intervals and filtered through a membrane filter (pore size: 0.45 μm). The BTMP concentration was determined using an UV spectrophotometer (Shimadzu UV-240, Kyoto, Japan) at 244 nm.

Release Study Mimicking the Chewing Action 1) The head of a disposable syringe (volume: 25 ml) was cut off and covered with thread netting (12 mesh). A definite weight (1.0 g) of SFG was inserted and pressed through the syringe into the dissolution test fluid (Fig. 1). The release behavior of BTMP from the slices of SFG (diameter: approx. 1 mm, length: approx. 12 mm) were determined by the JPXII paddle method (paddle rotating speed: 100 rpm) at 37 °C. The released BTMP was quantitatively analyzed as described. We termed this procedure the "press-through method."

2) We also used the "paddle-bead method" described by Aoki *et al.*[5)] These researchers modified the JPXII paddle method to produce a mechanical impact force during the dissolution. In this study, polystyrene beads (diameter: 6.5 mm, Wako Pure Chemical Industries., Ltd., Osaka, Japan) were added to the dissolution test medium; number of beads used was 2500. A 250 ml portion of distilled water (37° C) was used as the dissolution medium, and the paddle rotation speed was 25 rpm. A definite weight of cylindrical SFG was inserted into the dissolution medium, and the released BTMP was quantitatively analyzed (Fig. 2).

A 5.0 ml portion of the fluids was removed at certain intervals and the volume was kept constant by adding the same amount of dissolution medium at the same temperature.

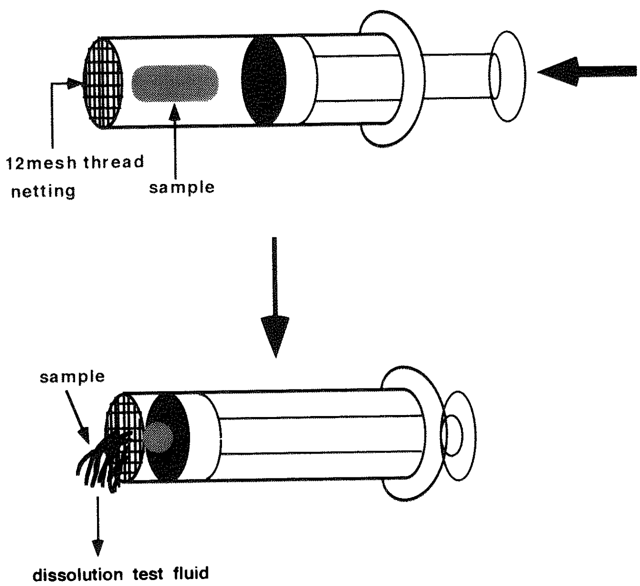

Fig. 1. Schematic View of Press-Through Method

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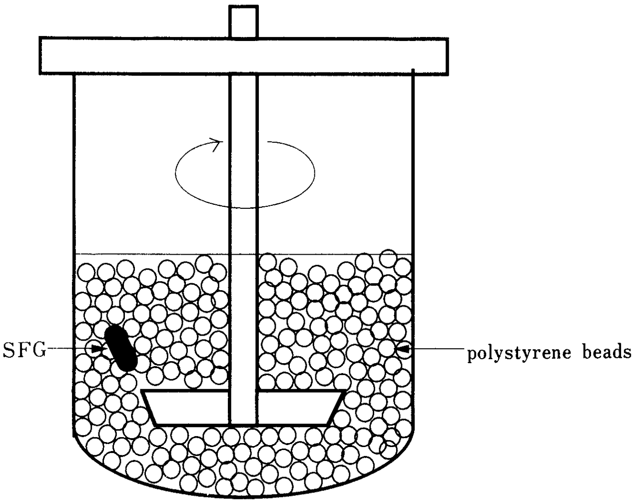

Fig. 2. Schematic Side View of Paddle Bead Method

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 placed in contact with the plunger, and the thickness of the SFG was measured. SFG was loaded at the rate of 1mm/s and the stress force and strain were measured at the moment of SFG breakage.

Solubility Study An excess amount of BTMP (200 mg: 4.29×10^{-4} mol) and successively increasing amounts of β-CD were added and dissolved in 10 ml of distilled water. The samples were shaken at 37 °C for 48 h. After equilibration, the samples were filtered with a Millipore filter (0.45 μm), and the amount of dissolved BTMP was spectrophotometrically analyzed at 244 nm.

Results and Discussion

Release of BTMP from the SFG Figure 3 shows the release profiles of BTMP from the SFG prepared at various concentrations of SF as determined by JP XII paddle method. The data shown were the average from triplicate experiments. No remarkable differences in the release profiles of BTMP from the SFGs containing 5 and 10% SF were observed. In contrast, the release rate from the SFG containing 20% SF was lower than the others, and the amount of BTMP released after 120 min was about eight-tenths that of the 5 or 10% SFG. To determine the effect of the presence of glycerol on the release profiles of BTMP from SFGs, various concentrations of glycerol solution were used as the solvent. Figure 4 shows these release profiles with and without glycerol as determined by JP XII paddle method. For SFG prepared with 10% glycerol and without glycerol, the release profiles were similar. A marked change of profiles was noted when the 50% glycerol solution was used as the solvent; the amount of BTMP released from the SFG prepared with 50% glycerol was about seven-tenths less than that of the others. Since no disintegration was seen throughout the dissolution test, in order to understand the mode of release of BTMP from SFG, the BTMP release data were analyzed using the relationship[6]:

$$M_t/M_\infty = k \cdot t^n \quad (1)$$

where M_t is the amount released at time t, M_∞ is the total amount released, t is the time (h) and k is a kinetic constant (h^{-n}). In this analysis, the release data at 240 min was adopted as the value of M_∞. The release exponent n characterizes the mechanism of release; the exponent takes the value of $n=0.5$ for Fickian diffusion, a value of $0.5<n<1.0$ for anomalous diffusion, and a value of $n=1$ for zero-order drug release. The values of the kinetic constant (k) and the diffusional exponents (n) are listed in Table 1. In the case of SFG prepared with 10 and 25% glycerol and without glycerol, a Fickian type diffusion was observed ($n=0.502$—0.503). In contrast, when the 50% glycerol was used as a solvent, the diffusion was non-Fickian, suggesting the anomalous nature of BTMP transport ($n=0.724$). On the basis of these results, the release rate of BTMP from SFG seems to be affected by addition of a high concentration of glycerol solution. Watanabe *et al.* demonstrated that the viscosity in the gel matrix is affected by adding sugar, and the migration rate of solute into and through the hydrogel matrix is retarded by the high viscosity of the fluid of interstices of the gel matrix.[7] In this study, the release properties of BTMP

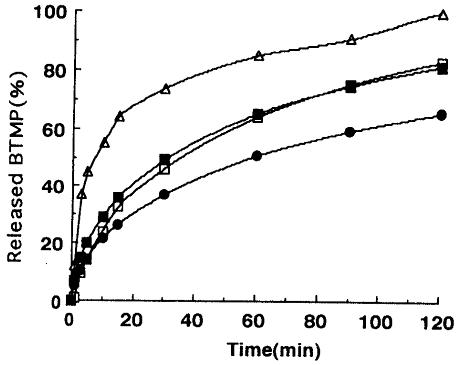

Fig. 3. Release Profiles of BTMP from SFG Prepared in Distilled Water Containing Various Amounts of SF

△, BTMP crystals; ■, SFG containing 5% SF; □, SFG containing 10% SF; ●, SFG containing 20% SF.

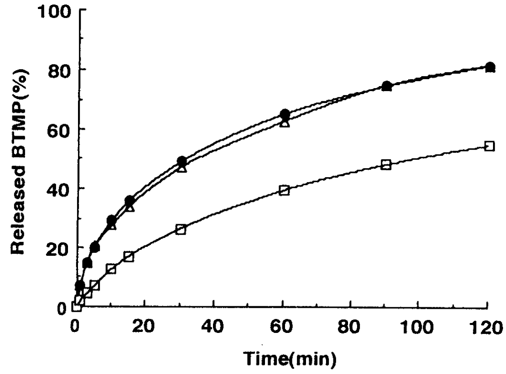

Fig. 4. Effects of Glycerol Content on the Release Profiles of BTMP from 10% SFG

●, SFG without glycerol; △, SFG prepared with 10% glycerol; □, SFG prepared with 50% glycerol.

Table 1. Fitting of BTMP Release Data to Eq. 1

Glycerol %	Kinetic constant k, h^{-n}	Release exponent n	Correlation coefficient r^2
0	0.614	0.503	0.985
10	0.798	0.502	0.992
25	0.787	0.503	0.992
50	0.754	0.724	0.992

from SFG seemed to be dependent upon the viscosity of the fluid of interstices of the SFG matrix, that is, the viscosity of the SFG was influenced by the glycerol concentration, so the release rate from the SFG might be reduced with an increase in glycerol concentration. Figure 5 shows the release profiles of BTMP from the SFGs prepared in 50% glycerol solution investigated by JPXII paddle method. The release rate of BTMP from SFGs decreased with an increase in SF content. This was explained by presuming the existence of a closely networked SFG at the higher SF concentrations. In practice, however, the BTMP is so weakly soluble in water that that used could not be completely dissolved in the SFG; thus SFG containing the homogeneously dispersed BTMP was not obtainable under these experimental conditions.

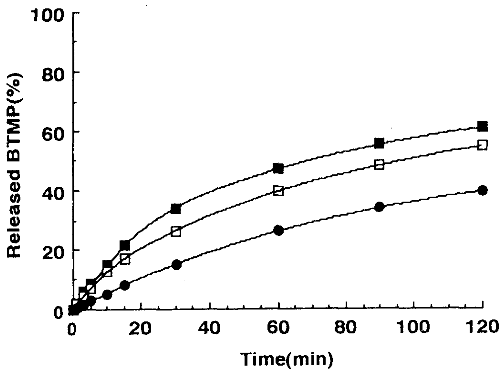

Fig. 5. Release Profiles of BTMP from SFG Containing Various Amounts of SF and Prepared with 50% Glycerol

■, SFG containing 5% SF; □, SFG containing 10% SF; ●, SFG containing 20% SF.

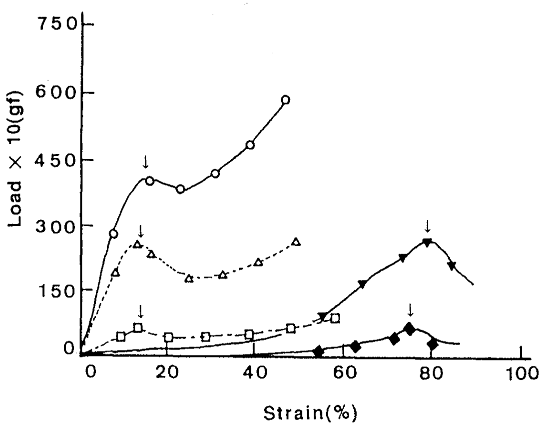

Fig. 6. Effects of the SF Content on the Rheological Properties of SFG Prepared with 50% Glycerol

□, SFG containing 5% SF; △, SFG containing 10% SF; ○, SFG containing 20% SF; ◆, 5% gelatin gel; ▼, 10% gelatin gel.

Rheological Study of SFG Elderly patients have great difficulty taking certain dosage forms because of impaired swallowing.[8] Therefore a certain fragility is necessary so that jelly-like preparations can be more easily swallowed. In a previous paper, we reported that the 5% SFG prepared in 50% glycerol solution showed such a fragility and breaking stress, similar to 5% commercial gelatin gel, meaning that the same texture as 5% gelatin gel is expected. In this study, the texture of SFGs containing BTMP prepared in 50% glycerol solution was evaluated in the same manner.[1] Figure 6 illustrates the stress–strain curves of SFG containing BTMP at various SF contents. The breaking point of each gel which denotes the firmness of SFG and the rupture that takes place at this point, is shown by the arrows. The load at breaking points increased with increase in SF content, and the SFGs containing 5.0 or 10.0% SF were similar to those of 5.0 or 10.0% gelatin gel normally used for commercial jelly. The gel breaking strain indicates the fragility of the material. In the SFG containing 5.0, 10.0 and 20.0% SF, the gel strains were as low as about 12.5%, that is about one-seventh lower than that of the 5 or 10% gelatin gels. These results show the same propensity as that of SFGs without drugs previously described, meaning the existence of BTMP does

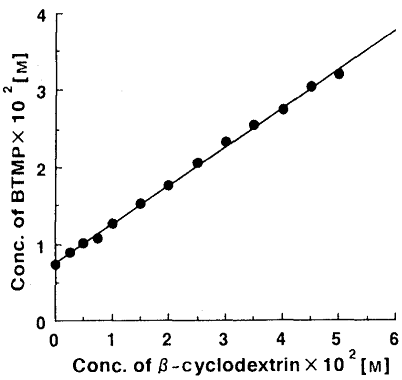

Fig. 7. Phase Solubility Diagram of BTMP and β-CD System in Water at 37°C

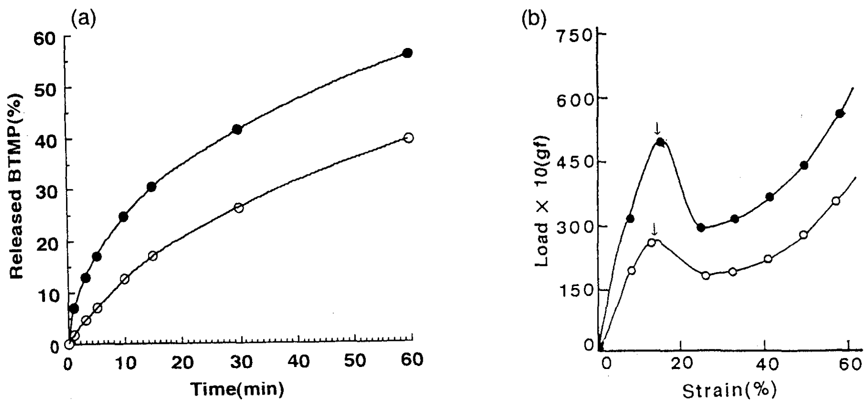

Fig. 8. Effects of the Existence of β-CD or Glycerol on the Release Profiles of BTMP from SFG Containing Various Amounts of SF

(a) Release profiles of BTMP. (b) Stress–strain curves of SFG. ●, SFG containing 10% SF (with β-CD); ○, SFG containing 10% SF (without β-CD).

not affect the rheological properties of SFGs.

Effect of CD Existence on the Release of BTMP from the SFG CDs are well known to form inclusion compounds and are used in various research fields.[9] In the pharmaceutical field, they are applicable for the enhancement of solubility and for the stabilization of drugs.[10,11] In this study, to improve the solubility of BTMP, β-CD was used as a solubilizer. To determine the interaction between BTMP and β-CD, a phase solubility diagram was prepared. Figure 7 shows the A_L type solubility curve[12] obtained for the BTMP and β-CD system at 37 °C. These results showed that the addition of β-CD is effective in improving the solubility of BTMP. Figure 8a shows the effects of the presence of β-CD in SFG on the release profiles of BTMP from the 10% SFG prepared in 50% glycerol solution. With the addition of β-CD, the released amount of BTMP from the SFG after 120 min was about 1.3 times higher than that of SFG without β-CD. This suggests that the increase in the amount of BTMP released is attributable to improvement in the solubility of BTMP by β-CD addition. Figure 8b shows the stress–strain curves of SFG containing 10% SF with and without β-CD. The breaking point of SFG with β-CD is about 2 times higher than that without β-CD. Oakenfull and Scott demonstrated that the rigidity of gelatin gel increased by adding sugar or polyols.[13] In this study, the reason for the increase of rigidity with the addition of β-CD is not yet fully understood but might be related to the production of a more extended gel network.

Release Study Mimicking the Chewing Action In practical use, SFG is swallowed after chewing. Supposing that SFG was destroyed when administered or swallowed, we evaluated the release profile of BTMP during chewing by two methods, the "press-through method" and the "paddle-bead method." In the former, a release test was performed after the destruction of SFG by pressing it through thread netting (12 mesh). The paddle-bead method described by Aoki *et al.*[5] incorporated a modification of the paddle method in JP XII which caused a mechanical impact force during the dissolution process. The paddle-bead method was originally used to evaluate the correlation between *in vivo* and *in vitro* release, and Aoki *et al.* demonstrated that the profile of the *in vitro* release using this technique at 25 rpm in 250 ml of medium containing 2500 beads was similar to that of the *in vivo* release under a fasted condition in dogs.[5] In this study, we employed this method to evaluate the correlation between the release profiles of BTMP and rheological properties of SFG. Figure 9 shows a comparison of the release profiles of BTMP from the SFG investigated using the JP XII paddle method, the press-through method and the paddle-bead method. In all three, BTMP was quickly released from the destroyed SFG. With the paddle-bead method, SFG was completely disintegrated, while with the press-through method, the size of SFG was made smaller by pressing it through the thread netting and no further disintegration was observed. The differences between these latter two methods can be attributed to the difference in disintegration features. Though detailed information on changes in the surface area of SFGs during experiments was not gained in the present study, the increase of release rate in the paddle-bead method seems due to attributable to the increase of surface area of SFG caused by the rupture among the polystyrene beads. The relationship between the release behavior of BTMP and the firmness of SFG was evaluated by assessing, the release behavior of BTMP from the SFG containing various amounts of SF using the paddle-bead method. Figure 10 shows these release profiles and the rheological properties of the SFGs. The release rate of BTMP from the SFGs decreased with increase in SF content, and is inversely proportional to

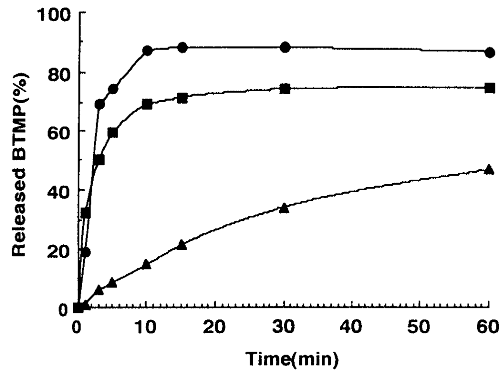

Fig. 9. Comparison of the Release Profiles of BTMP from SFG Investigated by Various Methods

●, paddle bead method; ■, press through method; ▲, JP XII paddle method.

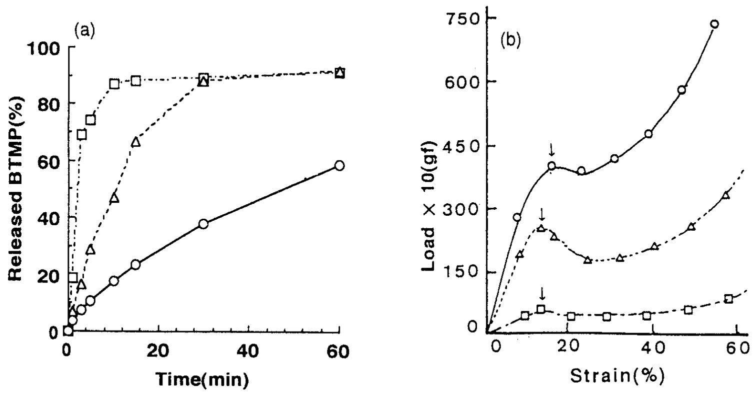

Fig. 10. Relationships between the Release Profiles of BTMP from SFG and the Rheological Properties of SFG

(a) Release profiles of BTMP from SFGs investigated by paddle-bead method. (b) Effects of the SF content on the rheological properties of SFG. □, SFG containing 5% SF; △, SFG containing 10% SF. ○, SFG containing 20% SF.

the firmness of SFGs observed in the rheological study. The rheological measurement showed that the SFG containing low SF content is so fragile and easily disintegrated that it may result in an increase in the release rate of BTMP.

In conclusion, the release behavior of BTMP from the SFG was affected by the SF and/or glycerol content. Addition of β-CD allowed complete dissolution of BTMP in the solvent, and there was both improvement in the release rate of BTMP from SFG and increase in the rigidity. The release rate of BTMP from SFG was, however, slower than the BTMP crystal. In this study, though the correlation between the release profiles observed with the paddle-bead method and *in vivo* release has not yet been identified, since the SFGs were disintegrated during the initial period of the paddle-bead method and BTMP was released simultaneously, the oral bioavailability of BTMP in practical use is apparently not influenced.

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