Effect of Formaldehyde on the Physicochemical Properties of Soft Gelatin Capsule Shells[1)]

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The effect of formaldehyde (FA) on three physicochemical properties of soft gelatin capsule shells was studied to interpret a change in the internal structure of those capsule shells whose disintegration time was remarkably prolonged by long-term storage at 40 °C or more. Shells with different degrees of crosslinking were prepared by immersing the shells in 0—3% FA solutions for 10 min. These shells were evaluated by measuring the following three physicochemical properties: equilibrium swelling ratio (S_{eq}), the gel strength of the swollen shells, and the percent of gelatin dissolved after 10 min (D_{10}) from the shells.

When the soft gelatin capsule shells were crosslinked by the treatment with FA, S_{eq} determined by second-order kinetics in the swelling of the shells decreased linearly with an increase in the amount of FA. The gel strength of the swollen shells increased with an increasing amount of FA, whereas the D_{10} of gelatin from the shells was diminished drastically at a given small amount of FA. From these findings, we confirmed that a three-dimensional network was formed tightly by interchain covalent crosslinking in the shells by treatment with FA.

These results were, on the whole, closely similar to the phenomenon observed in the disintegration test for the soft gelatin capsules stored long-term at 40 °C or more. Therefore, it was suggested that the evaluations of these physicochemical properties were available also for capsule shells treated by heating to clarify a mechanism for the prolongation of the disintegration time of the soft gelatin capsules.

Keywords soft gelatin capsule; crosslinking; formaldehyde; gelatin; disintegration; capsule shell

The disintegration time of soft gelatin capsules stored long-term at 40 °C is often increased beyond the limit (20 min) of the standard of the disintegration test in JP XII. We have observed this phenomenon and heretofore reported the effects of temperature,[2,3)] the moisture content of capsule shells[4)] and light[1)] on the disintegration time of the soft gelatin capsules. In conclusion, the disintegration time of only those capsules stored at 40 °C or more was found to be remarkably prolonged after a given storage time shorter than 6 months. Therefore, the storage condition of 40 °C and 75% relative humidity (40 °C, 75% R.H.), which is the standard condition for accelerated testing in Japan, was ascertained to be undesirable for that stability testing of the soft gelatin capsules.[2,5)]

The prolongation of the disintegration time of the soft gelatin capsules is possibly the result of a decrease in the solubility of gelatin crosslinked by heating. Generally, dehydrothermal treatment and UV irradiation are known as the physical methods of crosslinking. On the other hand, certain chemicals such as glutaraldehyde and formaldehyde (FA) are widely used as chemical crosslinking agents. In recent papers, Welz and Ofner[6)] reported on the drug release from gelatin film crosslinked by dehydrothermal treatment. Vandelli *et al.*[7)] clarified the effect of FA on the swelling properties and drug release of gelatin microspheres. Furthermore, Tomihata *et al.*[8)] compared the effect of various methods of crosslinking on the *in vitro* degradation of gelatin film by collagenase. In these studies, plain gelatin films or microspheres prepared in laboratories were employed as experimental samples. Little or no work has been reported on the physicochemical properties of the soft gelatin capsule shells prepared by an actual machine and treated with crosslinking agents.

In the present study, soft gelatin capsules containing medium-chain triglyceride, which is chemically inert to the capsule shells, were prepared using a rotary die process machine. These capsule shells were treated with FA, and model shells with different degrees of crosslinking were prepared. Three physicochemical properties which greatly affect the disintegration time of the soft gelatin capsules—the swelling kinetics of these shells, the gel strength of the swollen shells and the dissolution of gelatin from the shells—were evaluated in advance to clarify the change in the internal structure of the soft gelatin capsule shells stored at 40 °C or more.

Experimental

Materials The soft gelatin capsules (lot No. 4D274) containing about 300 mg of medium-chain triglyceride (Miglyol® 810, Mitsuba Trading Co.) as an inner fluid were prepared using a rotary die process machine (R. P. Scherer Co.). The size and shape of die pockets were No. 6 and oval, respectively. The pharmaceutical characteristics of these capsules are summarized in Table I. Formalin containing 37% FA (closslinking agent) and dichloromethane (washing solvent) were reagent grade commercial materials and were used without further purification. Gelatin employed in a protein assay was commercial type B gelatin (Nitta Gelatin Co.), and the lot No. (21214) of this gelatin was the same as that used

TABLE I. Pharmaceutical Characteristics of Soft Gelatin Capsule

Size and weight[a)]		Formula of capsule shell	
		Ingredient[b)]	Quantity (parts)
Length (mm)	12.67±0.03	Gelatin	100
Diameter (mm)	7.65±0.02	Concentrated glycerin	30
Capsule shell (mg)	127.1 ±0.8	Ethyl parahydroxybenzoate	0.4
Inner fluid[c)] (mg)	294.6 ±0.5	Propyl parahydroxybenzoate	0.1

a) Each value represents the mean±S.E. ($n=20$). *b*) All ingredients were JP XII grade. *c*) Miglyol® 810.

for the preparation of the soft gelatin capsules. For the determination of the concentration of dissolved gelatin, reagents A (lot No. 930416158) and B (lot No.930416156) as presented below (BCA protein assay reagent, Pierce Chemical Co.) were employed in this study. Reagent A contains sodium carbonate, sodium bicarbonate, bicinchoninic acid detection agent and sodium tartrate in 0.2 M NaOH, and reagent B is 4% copper sulfate solution. The lot Nos. of these reagents were kept constant all through the measurement in order to prevent variations in coloration.

Preparation of Sample A capsule shell was cut in half with a knife along the seam of the capsule. The halves of the shells were washed three times with dichloromethane to remove Miglyol® 810 from the shells, and were allowed to stand for 30 min at room temperature.

Crosslinking Treatment with FA 0—3% FA solutions were prepared by diluting formalin with pure distilled water. A weighed sample was immersed in 50 ml of each solution at 20 °C for 10 min. Each sample was removed from FA solution and its surface was quickly patted dry of the solution with lint-free tissues. It was then weighed, the amount of FA solution absorbed was determined, and the working amount of FA per 1 g of shell was calculated.

Evaluation of Swelling Kinetics Each sample crosslinked with FA was immersed in 50 ml of pure distilled water at 20 °C. At appropriate intervals for 50 min, the sample was weighed after gentle surface wiping using lint-free tissues and returned to its swelling water. The swelling ratio (S) was calculated using the following Eq. 1:

$$S=\frac{W_s-W_i}{W_i} \tag{1}$$

where W_i is initial weight of the sample and W_s is swollen weight of the same sample at immersion time (t) in pure distilled water. The swelling measurement was repeated three times.

Evaluation of Gel Strength Each sample crosslinked with FA was similarly immersed in 50 ml of pure distilled water at 20 °C for 10, 20 or 30 min. After gentle surface wiping, the gel strength of the swollen sample was determined using a rheometer (model NRM-2001J, Fudoh Kogyo Co.) equipped with a sharp-pointed needle (1.55 mm diameter × 42 mm) at room temperature under the following conditions: ascending rate of 20 mm/min; sensitivity of 200 g; chart speed of 20 mm/min. When the needle was thrust through the gel, a maximum force indicated on the chart was regarded as the gel strength of the swollen shells. The measurement was carried out five times because the values tended to vary widely.

Spectrophotometric Determination of Dissolved Gelatin 0.05—1.5 mg/ml of standard gelatin solutions were prepared using pure distilled water to prepare the standard curve for gelatin. The amount of gelatin was determined by colorimetric protein assay.[9] To prepare a working reagent, 2 ml of reagent B was mixed with 100 ml of reagent A. A 0.2 ml sample of each standard solution or pure distilled water was added to 4 ml of the working reagent in a test tube. Subsequent to sufficient mixing, these solutions were incubated at 37 °C for 30 min. After incubation, all tubes were cooled to room temperature, and the absorbance at 562 nm of each solution *vs.* water reference was measured using a spectrophotometer (model UV-2100, Shimadzu Seisakusho Co.).

Evaluation of Gelatin Dissolution A 100-ml Erlenmeyer flask containing 50 ml of pure distilled water as a dissolution medium was immersed in a thermoshaker (model TS-20, Toyo Kagaku Sangyo Co.) maintained at 37 °C.[10] After each sample treated with FA was placed in the medium, the flask was shaken at 100 strokes per minute and an amplitude of 30 mm for 30 min, and 0.2 ml of the medium was immediately collected at given intervals. The amount of gelatin dissolved in the medium was determined using the colorimetric protein assay described above. The measurement of gelatin dissolution was repeated three times.

Results and Discussion

Second-Order Kinetics in Swelling of Shells The soft gelatin capsule shells immersed in pure distilled water as the medium of the disintegration test in JP XII swelled gradually and eventually dissolved. However, as soon as the gelatin in the shells was crosslinked by any means, the swelling and dissolution of the shells was restrained rapidly. Thus, the rate at which the equilibrium swelling limit is reached, namely, the kinetics of the swelling, is an important factor in the disintegration of the soft gelatin capsules.

Schott[11] reported that the following second-order Eq. 2 was obeyed for the entire duration of the swelling of gelatin film in buffer solutions:

$$\frac{dS}{dt}=k(S_{eq}-S)^2 \tag{2}$$

where dS/dt is the rate of swelling at any given time (t), k is a specific rate constant for swelling, S_{eq} is an equilibrium swelling ratio and S is the swelling ratio. Integration of Eq. 2 results in:

$$S=\frac{kS_{eq}^2t}{1+kS_{eq}t} \tag{3}$$

Algebraic manipulation of Eq. 3 results in:

$$\frac{t}{S}=\frac{1}{kS_{eq}^2}+\frac{t}{S_{eq}} \tag{4}$$

Simplification of Eq. 4 by combining constant terms results in:

$$\frac{t}{S}=A+Bt \tag{5}$$

where A is $1/(kS_{eq}^2)$ and B is $1/S_{eq}$. Therefore, from the slope of a straight line plotted according to Eq. 5, the equilibrium swelling ratio, S_{eq}, is determined. This ratio is reached when the swelling pressure is equal to the elastic recovery tension of the swollen network.

The swelling of the shells is known to be influenced significantly by the osmotic pressure difference between capsule shell and solvent, the ionic strength, pH and temperature of the solvents. In this study, the same solvent as the medium (water) in the disintegration test was employed to interpret the disintegration of the soft gelatin capsules. The temperature of the solvent was maintained at 20 °C to prevent the significant dissolution of gelatin from the shells. This temperature is 14 to 16 °C below the gel melting point of aqueous gelatin gels of 17—24% solids.[11]

The increase in weight, representing imbibed water, was measured as a function of time and the swelling ratio *vs.* time curves were plotted. For instance, Fig. 1 shows the

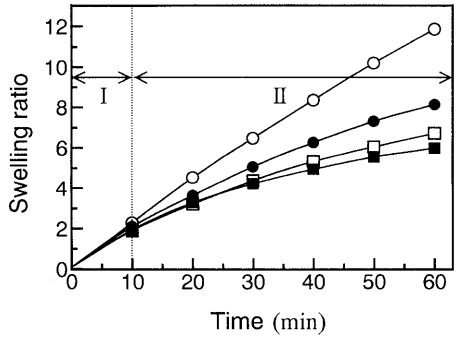

Fig. 1. Swelling Isotherm of Capsule Shell Treated with 0—3% Formaldehyde Solution

Medium: I, formaldehyde solution; II, pure distilled water. Concentration of formaldehyde: ○, 0%; ●, 1%; □, 2%; ■, 3%.

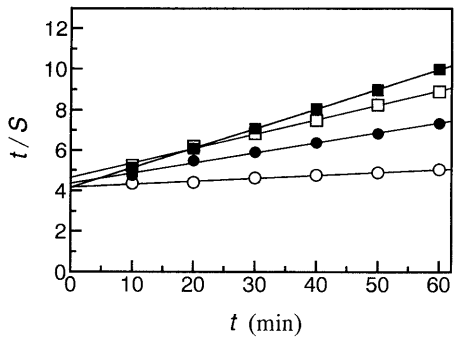

Fig. 2. Linear Regression of Swelling Isotherm of Fig. 1 According to Eq. 5

Concentration of formaldehyde: ○, 0%; ●, 1%; □, 2%; ■, 3%.

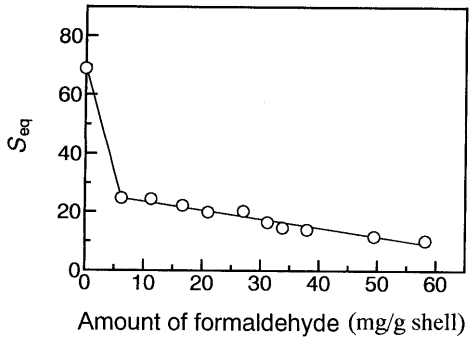

Fig. 3. Effect of Working Amount of Formaldehyde on Equilibrium Swelling Ratio (S_{eq}) of Capsule Shell

TABLE II. Equilibrium Swelling Ratio (S_{eq}) of Soft Gelatin Capsule Shell Treated with Formaldehyde

Formaldehyde (%)	$t/S=A+Bt$			S_{eq} (1/B)
	A	B	$r^{a)}$	
0	4.1847	0.0145	0.9916	69.01
0.25	3.5620	0.0404	0.9914	24.77
0.50	4.0320	0.0409	0.9995	24.43
0.75	4.1140	0.0449	0.9977	22.28
1.00	4.3667	0.0501	0.9970	19.94
1.25	4.2127	0.0493	0.9973	20.30
1.50	4.2687	0.0608	0.9992	16.45
1.75	4.5133	0.0681	0.9998	14.68
2.00	4.6507	0.0717	0.9983	13.94
2.50	4.2180	0.0856	0.9997	11.68
3.00	4.1640	0.0970	0.9999	10.31

a) Correlation coefficient.

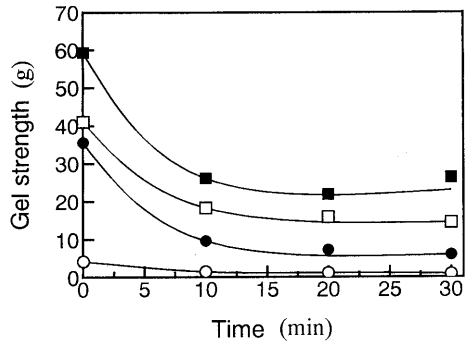

Fig. 4. Effect of Immersion Time on Gel Strength of Capsule Shell Treated with 0—3% Formaldehyde Solution

Concentration of formaldehyde: ○, 0%; ●, 1%; □, 2%; ■, 3%.

typical swelling isotherms of soft gelatin capsule shells treated with 0, 1, 2 or 3% FA solutions for 10 min. The swelling ratio increased with time, leveled off gradually in the case of the treatment with 2 and 3% FA solutions, and approached the equilibrium swelling ratio (S_{eq}) asymptotically. The isotherms of Fig. 1 were transformed into straight lines utilizing Eq. 5 (Fig. 2). The linear correlation coefficients of these lines exceeded 0.99. Therefore, from the reciprocal of the slopes of the lines, S_{eq} could be determined. These S_{eq} of the soft gelatin capsule shells crosslinked with 0—3% FA solutions for 10 min are summarized in Table II. Subsequently, these S_{eq} were plotted as a function of the working amount of FA per 1 g of the shell (Fig. 3). After S_{eq} decreased steeply initially, it decreased linearly with an increasing amount of FA according to the following Eq. 6:

$$S_{eq}=2.691\times 10-3.084\times 10^{-1}W \quad (n=10,\ r=0.9751) \tag{6}$$

where W is the working amount (mg) of FA per 1 g of the shell. FA is known to form methylene bonds to amino groups in gelatin according to the following Eq. 7[12]:

$$R\text{–}NH_2+HCHO+R\text{–}NH_2 \rightleftarrows R\text{–}NH\text{–}CH_2\text{–}NH\text{–}R+H_2O \tag{7}$$

The decrease in S_{eq} occurs because a three-dimensional network is formed tightly by interchain covalent crosslinking with an increase in the amount of FA. The diffusion of water into the shells is retarded, so that S_{eq} is decreased. Thus, it was clarified that the swelling of the capsule shells crosslinked with FA changed from infinite swelling into the limited swelling. From these results, we found that second-order kinetics was applicable to the swelling of the soft gelatin capsule shells crosslinked with FA, and that S_{eq} of these shells treated with a given amount of FA could be estimated utilizing Eq. 6.

Gel Strength of Swollen Shells Many methods of gel strength measurement have been reported.[13-16] None, however, was suitable for this study from the standpoint of a measurable range, accuracy and convenience. After treatment with a given amount of FA, the gel strength of the soft gelatin capsule shells swollen in pure distilled water showed fairly high value. Thus, the sharp-pointed needle, which makes it possible to determine the gel strength in a wide range, was employed in this study as a rheometer attachment.

Figure 4 shows the effect of immersion time on the gel strength of the soft gelatin capsule shells treated with 0, 1, 2 or 3% FA solutions for 10 min. In all cases, the gel strength decreased with immersion time and became approximately constant after 20 min. Therefore, the gel strength of the soft gelatin capsule shells immersed for 20 min was adopted as the representative value of the swollen shells for all subsequent evaluations. Figure 5 shows the effect of the amount of FA on the gel strength of the swollen shells. The gel strength increased almost linearly with increasing amounts of FA. This finding demonstrates directly the formation of the tight three-

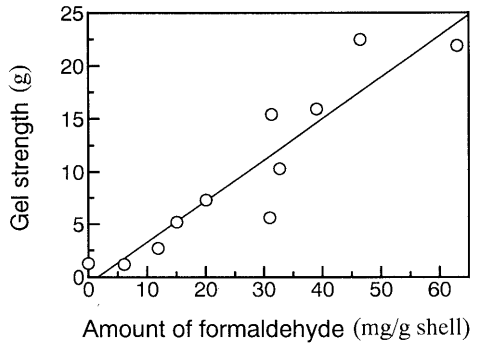

Fig. 5. Effect of Working Amount of Formaldehyde on Gel Strength of Capsule Shell Immersed in Water for 20 min

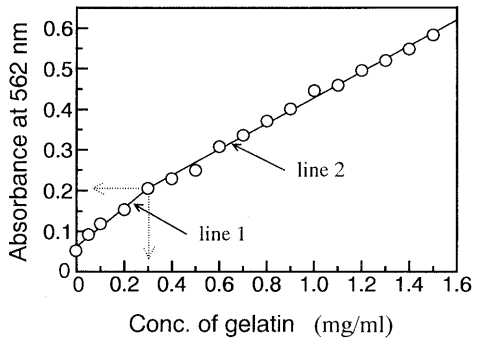

Fig. 6. Relationship between Absorbance at 562 nm and Concentration of Gelatin

dimensional network as described above.

Dissolution of Gelatin from Shells Several studies[6,17] have been reported on the dissolution characteristics of gelatin. Few works, however, have been published on those of gelatin from soft gelatin capsule shells. Hom *et al.*[18] determined indirectly the dissolution profiles of capsule shells containing FD & C Blue No.1 by the assay of this pigment instead of gelatin. In addition, we applied the procedure developed by Smith *et al.*[9] to the present study to determine directly the amount of gelatin dissolved from the capsule shells.

Figure 6 shows the standard curve for gelatin in this method. As can be seen, this curve did not exactly follow Beer's law, which was also the case in the above literature.[9] However, it is nonetheless adequate for measuring gelatin concentration in a wide range of potential samples. The following Eqs. 8 and 9 were obeyed for the relationship between the absorbance and the concentration of gelatin over the range of concentrations from 0 to 0.3 mg/ml and 0.3 to 1.5 mg/ml, respectively:

$$C = 6.212 \times 10^{-2} + 4.822 \times 10^{-1} A \quad (n=5,\ r=0.9912) \tag{8}$$

$$C = 1.086 \times 10^{-1} + 3.200 \times 10^{-1} A \quad (n=13,\ r=0.9973) \tag{9}$$

where C is the concentration of gelatin and A is the absorbance at 562 nm. These lines gave a linear relationship with excellent correlation coefficients. Thus, when the absorbance of gelatin solutions was below 0.21 and 0.21 or above, the concentrations of gelatin were determined utilizing Eqs. 8 (line 1) and 9 (line 2), respectively. FA

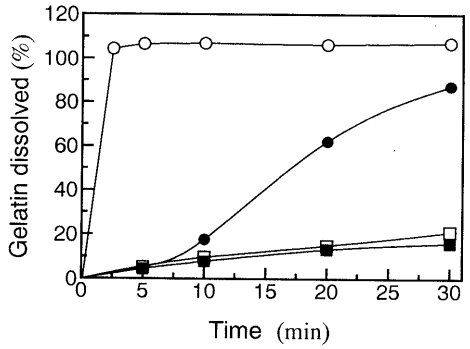

Fig. 7. Dissolution Profile of Gelatin from Capsule Shell Treated with 0—3% Formaldehyde Solution

Concentration of formaldehyde: ○, 0%; ●, 1%; □, 2%; ■, 3%.

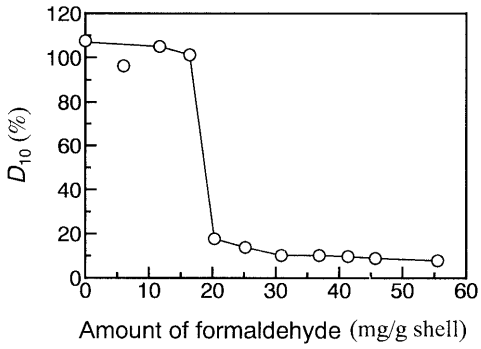

Fig. 8. Effect of Working Amount of Formaldehyde on Percent of Gelatin Dissolved after 10 min (D_{10}) from Capsule Shell

was previously confirmed not to interfere with the colorimetric protein assay.

Figure 7 shows the dissolution profiles of gelatin from the soft gelatin capsule shells treated with 0, 1, 2 or 3% FA solutions for 10 min. The rate and amount of gelatin dissolved from the shells decreased with increasing concentrations of FA. In the case of treatment with 0% FA solution (pure distilled water), the rate of dissolution of gelatin from the shells was very fast: about 100% of the gelatin was dissolved after 5 min. From this, it was confirmed that gelatin was dissolved completely after 10 min. Therefore, the percentage dissolved after 10 min (D_{10}) was adopted as a parameter for all subsequent evaluations for the capsule shells treated with FA.

Figure 8 shows the effect of the working amount of FA on the D_{10} of gelatin from the shells. D_{10} did not change significantly up to 16.4 mg/g shell of the working amount of FA; however, it began to decrease rapidly after the point of 20.3 mg/g shell. Subsequent to this significant lowering, D_{10} decreased gradually with increasing amounts of FA. Finally, D_{10} closely approached 0% at the point of about 310 mg/g shell (data not shown). On the other hand, it was observed visually that the capsule shells whose D_{10} decreased below 10% maintained a swollen state at 37 °C for 30 min in a test fluid and varied into the insoluble shells. These experimental results demonstrate that treatment with only 20.3 mg/g shell of FA, which results from the treatment with 1% FA solution at 20 °C for 10 min, was sufficient to prevent the dissolution of gelatin in water from the soft gelatin capsule

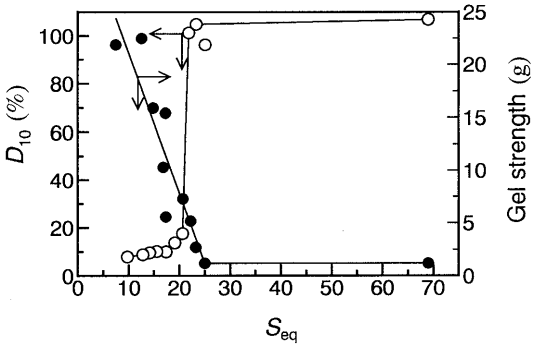

Fig. 9. Relationship between D_{10} of Gelatin from Capsule Shell or Gel Strength of Capsule Shell Immersed in Water for 20 min and S_{eq}

○, D_{10}; ●, gel strength.

shells.

Relationships between D_{10} or Gel Strength and S_{eq} Figure 9 illustrates the relationships between D_{10} of gelatin from the capsule shells or the gel strength of the swollen shells and S_{eq}. D_{10} increased drastically from 18 to 101% with a slight increase (from 20.7 to 21.9) in S_{eq}. This result indicates the existence of some critical equilibrium swelling ratio between 20.7 and 21.9 at which the D_{10} of gelatin from the capsule shells increases dramatically. The gel strength decreased linearly with an increase in S_{eq} up to the point of 25.03, and thereafter it did not change, maintaining a sufficiently low value.

These relationships are easily presumed from the result shown in Fig. 3. However, in these relationships the working amount of FA is not involved as a factor. Therefore, it is considered that the evaluation of these relationships is useful for versatile comparisons of changes in the internal structure of the capsule shells treated by heating with that of the shells treated with FA.

Conclusion

When the soft gelatin capsule shells were crosslinked by the treatment with FA, the equilibrium swelling ratio of the soft gelatin capsule shells decreased linearly with increasing amounts of FA. This is because the diffusion of water into the shells was retarded by the formation of the tight network. The gel strength of the swollen shells increased with an increase in the amount of FA. This demonstrates directly that a three-dimensional network is formed tightly by crosslinking. The dissolution of gelatin from the shells was found to be prevented remarkably at a given working amount of FA because of the effective combination of covalent intramolecular linkages.

These results were, on the whole, closely similar to the phenomenon observed in the disintegration test for the soft gelatin capsules stored at 40 °C or more for long-term.[2-4] Therefore, it was suggested that the evaluation of the physicochemical properties as described above were available to the capsule shells treated by heating to clarify a mechanism for the prolongation of the disintegration time of the soft gelatin capsules.

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