

## Effect of Storage Temperature on the Physicochemical Properties of Soft Gelatin Capsule Shells<sup>1)</sup>

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The effect of storage temperature on the 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 storage at 40 °C or more. The capsules were stored at 25, 40 or 60 °C for a maximum of 6 months. The time-courses of the disintegration time of these capsules were determined and compared with those of the following three physicochemical properties of the shells: the 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. From these evaluations, it was found that the prolongation of the disintegration time of the capsules stored at 40 °C was based on a decrease in  $S_{eq}$  of the shells and/or  $D_{10}$  of gelatin from the shells, and/or an increase in the gel strength of the swollen shells.

In addition, these physicochemical properties of the shells stored at different temperatures were compared with those of shells treated with formaldehyde. The three properties of the shells stored at 40 °C for 6 months were, on the whole, similar to those of the shells treated with a 1% formaldehyde solution. Furthermore, in the relationships between  $D_{10}$  or gel strength, and  $S_{eq}$ , no fundamental difference was observed between the shells stored at various temperatures and those treated with formaldehyde.

Thus, we demonstrated that the prolongation of the disintegration time of the soft gelatin capsules stored at 40 °C or more is brought about by a change in the internal structure of the capsule shells in a manner similar to the case of shells treated with formaldehyde.

**Keywords** soft gelatin capsule; disintegration; cross-linking; gelatin; capsule shell; storage

Soft gelatin capsules have a multitude of applications and advantages over conventional dosage forms such as tablets, hard gelatin capsules, granules, solutions, etc. These advantages include increased bioavailability for poorly water soluble drugs,<sup>2-4)</sup> good stability,<sup>5,6)</sup> good content uniformity<sup>7)</sup> and easy processibility.<sup>8)</sup> Soft gelatin capsules can also be used for the delivery of therapeutic materials that are liquid in nature, those that pick up moisture during processing, or those that pose problems during compression.<sup>9)</sup> As a disadvantage, the disintegration time of the soft gelatin capsules stored at 40 °C for 6 months is often increased beyond the limit (20 min) of the standard disintegration test described in JP XII in accelerated testing for these capsules.<sup>10-14)</sup> Therefore, in evaluating the stability of soft gelatin capsules, particular attention should be given to the storage temperature.

In our previous paper,<sup>1)</sup> the effect of formaldehyde on the following three physicochemical properties of soft gelatin capsule shells was studied in advance to interpret a change in the internal structure of the capsule shells stored at 40 °C: equilibrium swelling ratio, gel strength of the swollen shells, and percent of gelatin dissolved after 10 min from the shells. From these evaluations, a three-dimensional network was confirmed to be tightly formed by interchain covalent cross-linking in the shells by treatment with formaldehyde. We reported, in conclusion, that the evaluation of these physicochemical properties may apply also to capsule shells treated by heating.

In this study, soft gelatin capsules containing only medium-chain triglyceride, which had been employed in the previous study,<sup>1)</sup> were again used. The time-course of disintegration time of the capsules and the three physicochemical properties of the shells stored at 25, 40

and 60 °C for a maximum of 6 months were determined. The changes in these properties of those shells treated by heating were compared with those of the shells treated with formaldehyde to clarify the mechanism by which the disintegration time of the soft gelatin capsules is prolonged.

### Experimental

**Materials** The soft gelatin capsules employed in the present study were identical with those used in the previous study.<sup>1)</sup> That is, these capsules contained about 300 mg of medium-chain triglyceride (Miglyol® 810, Mitsuba Trading Co.) as an inner fluid, and were prepared using a rotary die process machine (R. P. Scherer Co.). The size and shape of the die pockets were No. 6 and oval, respectively. Dichloromethane as a washing solvent was reagent grade commercial material and was used without further purification. The gelatin employed in a protein assay was commercial type B gelatin (lot No. 21214, Nitta Gelatin Co.) and the lot No. of this gelatin was the same as that used for the preparation of the soft gelatin capsules. For the determination of the concentration of dissolved gelatin, the same BCA protein assay reagents (Pierce Chemical Co.) as described in the previous paper<sup>1)</sup> were employed. The lot Nos. of reagents A and B were 930104097 and 921228083, respectively, throughout the measurement in order to prevent variations in coloration.

**Storage of Capsules** 50 capsules were placed in a 50-ml amber glass bottle (JIS No. 5), and each bottle was stoppered to prevent the absorption or desorption of water. These glass bottles containing the soft gelatin capsules were stored in incubators adjusted to 25, 40 or 60 °C for a maximum of 6 months. In the case of the storage at 60 °C, the individual capsules were wrapped with aluminium foil to prevent adhesion between the capsules.

**Evaluation of Disintegration Time** The disintegration time of the capsules in water was measured using an apparatus for disintegration testing (model T-6H, Toyama Sangyo Co.) according to JP XII. The measurement was carried out for 12 capsules.

**Preparation of Sample**<sup>1)</sup> 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 they were allowed to stand for 30 min at room temperature. The average thickness and moisture content of the shells were 0.89 mm and

9.3%, respectively.

**Evaluation of Swelling Kinetics<sup>1)</sup>** Each sample stored at various temperatures was immersed in 50 ml of pure distilled water at 20 °C. At 10 min intervals for 60 min, each 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:

$$S = \frac{W_s - W_i}{W_i} \quad (1)$$

where *W<sub>i</sub>* is the initial weight of the sample and *W<sub>s</sub>* is the 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<sup>1)</sup>** Each sample 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. The measurement was carried out five times because the values tended to vary widely.

**Spectrophotometric Determination of Dissolved Gelatin<sup>1)</sup>** 0.05–1.4 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 a colorimetric protein assay<sup>15)</sup> according to the previous paper.<sup>1)</sup> A 0.2 ml sample of each standard solution or pure distilled water was added to 4 ml of a working reagent (the mixture of 50 parts of reagent A and 1 part of reagent B) 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<sup>1)</sup>** 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. After each sample was placed in the medium, the flask was shaken at 100 strokes per minute and at 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.

**Measurement of Loss on Drying** The moisture content of the capsule shells varied under the influence of environmental humidity. The loss on drying of about 300 mg of the capsule shells at 105 °C for 2 h was measured in each case to correct the percentage of dissolved gelatin.

**Results and Discussion**

**Disintegration Time of Capsules** Figure 1 shows the time-course of the disintegration time of the soft gelatin capsules stored at various temperatures. In Fig. 1, a dotted line represents the limit (20 min) of the standard of the disintegration test in JP XII. The disintegration time of the capsules stored at 60 °C was increased beyond the limit within a week. That of the capsules stored at 40 °C was increased with the storage time, and exceeded the limit at 6 months. However, a significant change in the disintegration time was not observed for the capsules stored at 25 °C for 6 months. From these findings, we reconfirmed that the disintegration time of the soft gelatin capsules stored at 40 °C or more for 6 months is remarkably prolonged beyond the limit of the standard.<sup>10–12)</sup>

**Second-Order Kinetics in Swelling of Shells** Figure 2 shows the swelling isotherms of the soft gelatin capsule shells stored at 25 or 40 °C for 6 months. The swelling ratio increased with the time, leveled off gradually, and tended to approach an equilibrium value (the equilibrium swelling ratio) asymptotically, as in the case of shells treated with formaldehyde.<sup>1)</sup> From these results, it was considered that the following second-order Eq. 2<sup>16)</sup> was

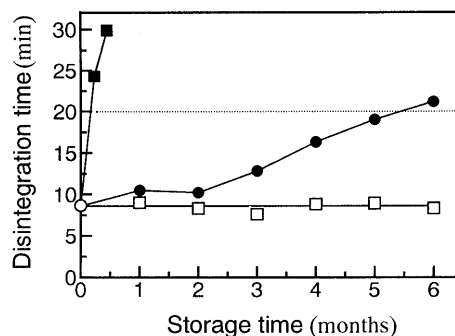


Fig. 1. Time-Course of Disintegration Time of Capsule Stored at Various Temperatures

Storage temperature: ○, initial; □, 25 °C; ●, 40 °C; ■, 60 °C. A dotted line represents the limit (20 min) of the standard of the disintegration test in JP XII.

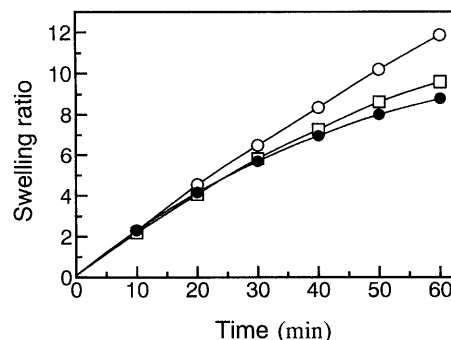


Fig. 2. Swelling Isotherm of Capsule Shell Stored at 25 or 40 °C for 6 Months

Storage temperature: ○, initial; □, 25 °C; ●, 40 °C.

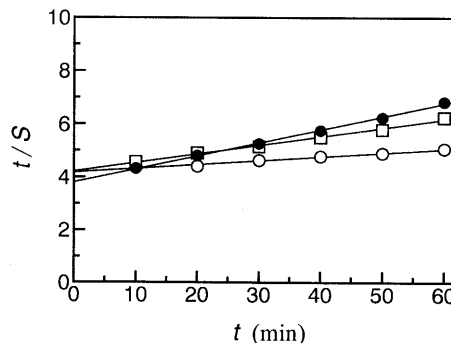


Fig. 3. Linear Regression of Swelling Isotherm of Fig. 2 According to Eq. 3

Storage temperature: ○, initial; □, 25 °C; ●, 40 °C.

also obeyed for the entire duration of the swelling of these capsule shells in water:

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

where *dS/dt* is the rate of swelling at any given time (*t*), *k* is a specific rate constant for swelling, *S<sub>eq</sub>* is the equilibrium swelling ratio and *S* is the swelling ratio. Conversion of Eq. 2 results in<sup>1)</sup>:

$$\frac{t}{S} = A + Bt \quad (3)$$

where *A* is 1/(*kS<sub>eq</sub><sup>2</sup>*) and *B* is 1/*S<sub>eq</sub>*. The isotherms of Fig. 2 were transformed into straight lines utilizing Eq. 3 in Fig. 3. These lines gave linear relationships with excellent

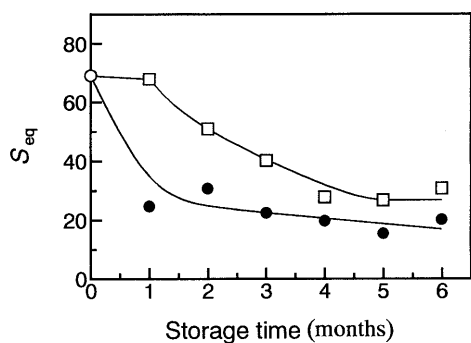


Fig. 4. Time-Course of Equilibrium Swelling Ratio ( $S_{eq}$ ) of Capsule Shell Stored at 25 or 40°C

Storage temperature: ○, initial; □, 25°C; ●, 40°C.

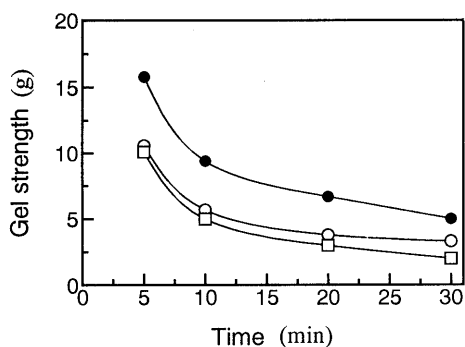


Fig. 5. Effect of Immersion Time on Gel Strength of Capsule Shell Stored at 25 or 40°C for 6 Months

Storage temperature: ○, initial; □, 25°C; ●, 40°C.

correlation coefficients. Therefore, from the slope of these lines,  $S_{eq}$  could be determined.

Figure 4 shows the time-courses of  $S_{eq}$  of the capsule shells stored at 25 or 40°C for 6 months.  $S_{eq}$  of the shells stored at 25°C was not significantly changed for a month. However, it decreased with the storage time, and approached a constant value after 4–5 months. On the other hand,  $S_{eq}$  of the shells stored at 40°C decreased rapidly for a month; subsequently, it decreased gradually with time. This decrease in  $S_{eq}$  is because the three-dimensional network is formed tightly and the diffusion of water into the shells is retarded. Therefore, in the case of the storage, particularly at 40°C, gelatin in the capsule shells was suggested to be cross-linked by heating.  $S_{eq}$  of the shells stored at 60°C could not be determined because the capsule shells became thin by lengthening.

**Gel Strength of Swollen Shells** Figure 5 shows the effect of immersion time on the gel strength of the soft gelatin capsule shells stored at 25 or 40°C for 6 months. At any storage temperature, the gel strength of the swollen shells decreased with immersion time. The gel strength of the shells stored at 25°C for 6 months was very close to that of the initial shells at any immersion time, whereas that of the shells stored at 40°C for the same period was significantly higher than that of the initial shells. To clarify the time-course of the gel strength, the strength of the shells immersed in water for 20 min, as described in the previous paper,<sup>1)</sup> was plotted against the storage time in Fig. 6. The gel strength of the shells stored at 25°C tended

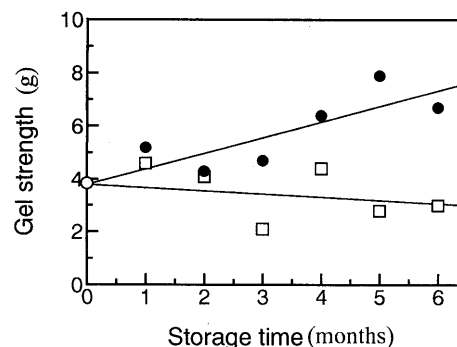


Fig. 6. Time-Course of Gel Strength of Capsule Shell Stored at 25 or 40°C

Storage temperature: ○, initial; □, 25°C; ●, 40°C. Each value represents the gel strength of the swollen capsule shells immersed in water for 20 min.

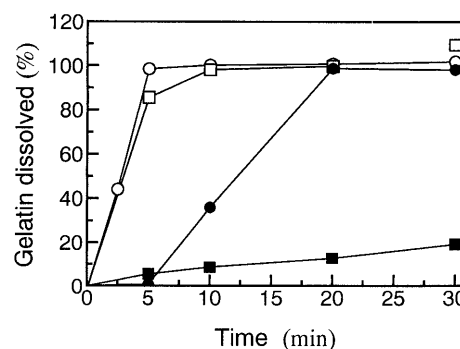


Fig. 7. Dissolution Profile of Gelatin from Capsule Shell Stored at Various Temperatures for 6 Months

Storage temperature: ○, initial; □, 25°C; ●, 40°C; ■, 60°C. In the case of the storage at 60°C, the storage period is 6 weeks.

to decrease slightly with the storage time. However, taking into consideration experimental errors, it seems reasonable to assume that the gel strength of the swollen shells stored at 25°C does not vary in the period ranging 0 to 6 months. On the other hand, the gel strength of the shells stored at 40°C increased distinctly with the time. This finding demonstrates directly the formation of the tightly three-dimensional network in almost the same manner as in the case of the shells treated with formaldehyde.<sup>1)</sup>

**Dissolution of Gelatin from Shells** Figure 7 shows the dissolution profiles of gelatin from soft gelatin capsule shells stored at various temperatures for 6 months (at 25 or 40°C) or 6 weeks (at 60°C). The rate and amount of gelatin dissolved from the shells decreased with a rise in temperature. Figure 8 shows the time-course of the percent of gelatin dissolved after 10 min ( $D_{10}$ ) from shells stored at various temperatures.  $D_{10}$  of gelatin from the shells stored at 60°C fell below 10% after 2 weeks, and it was not changed by 6 weeks. We observed, then, that these shells stored at 60°C for 2–6 weeks maintained a swollen state for 30 min in a test fluid and varied into the insoluble shells in a manner similar to the case of the shells treated with formaldehyde.<sup>1)</sup>  $D_{10}$  of gelatin from the shells stored at 40°C did not vary for 4 months; however, it began to decrease suddenly from the point of 5 months. By contrast, the  $D_{10}$  from the shells stored at 25°C was maintained at 100% for 6 months. These findings strongly suggest that gelatin in the soft gelatin capsule shells is cross-linked

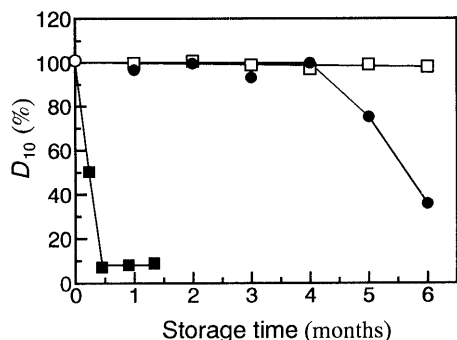


Fig. 8. Time-Course of Percent of Gelatin Dissolved after 10 min ( $D_{10}$ ) from Capsule Shell Stored at Various Temperatures

Storage temperature: ○, initial; □, 25°C; ●, 40°C; ■, 60°C.

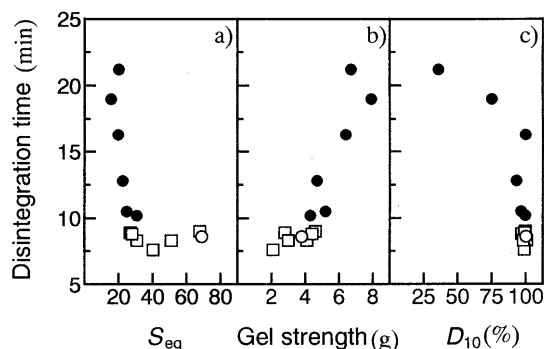


Fig. 9. Effect of  $S_{eq}$  (a), Gel Strength (b) or  $D_{10}$  (c) of Capsule Shell on Disintegration Time of Capsule Stored at 25 or 40°C for Various Periods

Storage temperature: ○, initial; □, 25°C; ●, 40°C.

with storage time by heating at 40°C or more.

**Effect of Physicochemical Properties of Shells on Disintegration Time of Capsules** Figure 9 shows the effects of  $S_{eq}$ , the gel strength or  $D_{10}$  of the soft gelatin capsule shells on the disintegration time of the capsules stored at 25 or 40°C for various periods. From these relationships, it seems that the disintegration time of the capsules increased in proportion to the gel strength of the shells, and in inverse proportion to  $S_{eq}$  of the shells or  $D_{10}$  of gelatin. Thus, the ratio ( $R$ ) of the gel strength to the product of  $S_{eq}$  and  $D_{10}$  (%) was defined in the following Eq. 4:

$$R = \frac{I_g}{S_{eq} \cdot D_{10}} \quad (4)$$

where  $I_g$  (g) is the gel strength of the swollen shells immersed in water for 20 min. In order to obtain the natural logarithmic values of  $R$  of 0 or above, the ratio ( $R_{ph}$ ) of the physicochemical properties of the capsule shells was defined in the following Eq. 5:

$$R_{ph} = \frac{1 \times 10^4 \cdot I_g}{S_{eq} \cdot D_{10}} \quad (5)$$

The effect of the natural logarithmic value of  $R_{ph}$  on the disintegration time of the capsules is shown in Fig. 10. The disintegration time was not significantly changed over the  $\ln R_{ph}$  range of 1.66–2.30; however, it began to increase linearly with increasing  $\ln R_{ph}$  in the range of 2.30–4.52 according to the following Eq. 6:

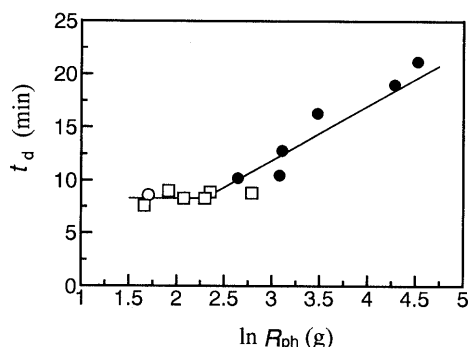


Fig. 10. Effect of Ratio of Physicochemical Properties ( $R_{ph}$ ) Defined in Eq. 5 on Disintegration Time ( $t_d$ ) of Capsule Stored at 25 or 40°C for Various Periods

Storage temperature: ○, initial; □, 25°C; ●, 40°C.

$$t_d = -5.779 + 5.885 \cdot \ln R_{ph} \quad (6)$$

( $n=9, r=0.970$ )

where  $t_d$  is the disintegration time of the capsules. An initial increase in  $\ln R_{ph}$  observed in Fig. 10, which was merely based on a decrease in  $S_{eq}$  of the shells, did not influence the disintegration time of the capsules. However, accompanied by a decrease in  $S_{eq}$  and  $D_{10}$ , and an increase in the gel strength, when  $\ln R_{ph}$  increased beyond the point of 2.30, it began to strongly affect the disintegration time of the capsules. These findings plainly indicate that the prolongation of the disintegration time of the capsules stored at 40°C resulted from a decrease in the swelling of the shells and/or the dissolution of gelatin, and/or an increase in the gel strength of the swollen shells. The obvious level of the effect of each physicochemical property of the shells on the disintegration time of the capsules could not be determined in the present study. However, it seems reasonable to consider that the prolongation of the disintegration time of the soft gelatin capsules is based on a combination of the three effects described above.

**Comparison of Physicochemical Properties of Shells with Those of Shells Treated with Formaldehyde** To interpret the extent of cross-linking of gelatin, the physicochemical properties of the soft gelatin capsule shells stored at 40°C for 6 months, where the disintegration time of the capsules exceeds 20 min, were compared with those of the shells treated with formaldehyde. The physicochemical properties of the latter, which have already been reported in the previous paper,<sup>1)</sup> are summarized in Table I, together with the properties of the former shells treated by heating. From these data, we revealed that the three physicochemical properties of the soft gelatin capsule shells stored at 40°C for 6 months were, on the whole, similar to those of the shells treated with 1% formaldehyde solution at 20°C for 10 min.

On the other hand, two types of relationships between  $D_{10}$  of gelatin or the gel strength of the swollen shells, and  $S_{eq}$  of the shells were determined in the previous study<sup>1)</sup> to interpret the change in the internal structure of the capsule shells treated with formaldehyde. Thus, these relationships for the shells treated by heating were also finally evaluated in the present study. Figure 11 shows these relationships for the capsule shells stored at 25 or 40°C for various periods. In Fig. 11, the dotted curves

TABLE I. Physicochemical Properties of Capsule Shell Treated with Various Concentrations of Formaldehyde

Conc. of FA <sup>a)</sup>	$S_{eq}$	Gel strength (g)	$D_{10}$ (%)
(40 °C, 6 months <sup>b)</sup>	20.26	6.7	35.9)
0	69.01	1.2	106.9
0.25	24.77	1.2	96.3
0.50	24.43	2.7	105.0
0.75	22.28	5.2	101.3
1.00	19.94	7.3	17.7
1.25	20.30	5.6	13.8
1.50	16.45	10.3	10.1
1.75	14.68	15.4	10.2
2.00	13.94	15.9	9.7
2.50	11.68	22.5	8.9
3.00	10.31	21.9	7.9

a) Concentration of formaldehyde. b) Capsule shell stored at 40 °C for 6 months.

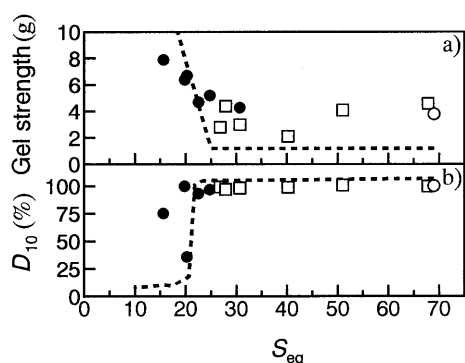


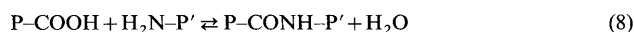
Fig. 11. Relationship between Gel Strength (a) or  $D_{10}$  (b) and  $S_{eq}$  of Capsule Shell Stored at 25 or 40 °C for Various Periods

Storage temperature: ○, initial; □, 25 °C; ●, 40 °C. Dotted curves represent the relationships for the capsule shells treated with formaldehyde described in the previous paper.<sup>1)</sup>

represent the relationships for the shells treated with formaldehyde as described in the previous paper.<sup>1)</sup> Formaldehyde is known to form methylene bonds between amino groups on a gelatin chain (P) and an adjacent chain (P') according to the following Eq. 7<sup>17)</sup>:



On the other hand, it has been reported that self-cross-linking of gelatin is induced by heating owing to a condensation reaction between a carboxyl group on a gelatin chain and an amino group on an adjacent chain according to the following Eq. 8<sup>18)</sup>.



In this way, the cross-linking mechanism of gelatin treated by heating is distinct from that treated with formaldehyde. But, Fig. 11 demonstrates that no fundamental difference is observed in the  $S_{eq}$  dependence of  $D_{10}$  of gelatin and the gel strength between the capsule shells treated with formaldehyde and those treated by heating. Therefore, the change in the internal structure of the soft gelatin capsule

shells stored at 40 °C or more was considered to be substantially the same as that of the shells treated with formaldehyde, in spite of the different cross-linking mechanism.

We have heretofore reported the effects of all environmental factors, except oxygen and micro-organisms, on the disintegration time of the soft gelatin capsules.<sup>10-12,19)</sup> In this series of studies, it is subsequently worth noting that the disintegration time of the capsules with desiccants is not remarkably prolonged for 8 months, in spite of the storage at 40 °C. If three physicochemical properties of the shells stored at 40 °C and desiccated with the desiccants are likewise evaluated and compared with those properties described above, the effect of moisture content of the shells on the change in the internal structure of those shells will be further clarified.

### Conclusion

We demonstrated that the prolongation of the disintegration time of the soft gelatin capsules stored at 40 °C or more is brought about by a change in the internal structure of the capsule shells in a manner similar to the case of shells treated with formaldehyde. Both these changes resulted from the cross-linking of gelatin in the capsule shells.

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