Regular Article

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Gelation Factors of Pectin for Development of a Powder Form of Gel, Dry Jelly, as a Novel Dosage Form

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Jellies for oral administration are dosage forms that contain water, as stipulated in the Japanese Pharmacopeia, and heat is generally applied to the jellies during the manufacturing process. Therefore, it is difficult to formulate drugs that may be affected adversely by water and/or heat. To solve this problem, we tried to develop a powder form of gel as a novel dosage form (dry jelly: jelly medicine extemporaneously prepared) that is converted to jelly after addition of water at the time of administration. For this purpose, a basic gel formulation consisting of pectin, glucono-δ-lactone, dibasic calcium phosphate hydrate, and sucrose was investigated to evaluate the critical factors affecting gelation phenomena. The gel form was developed by adjusting the amount of each component of the formulation and of water added. Gelation occurred even with hard water containing metal ions (hardness of approximately 304 mg/L), and no changes in gel hardness occurred. The desired gel hardness could be controlled by adjusting the amount of water. The gel hardness changed over time after the addition of water, but this change did not affect the dissolution behavior of drugs formulated in the dry jelly.

Key words dry jelly; jelly medicine extemporaneously prepared; gelation; pectin; calcium

Pneumonia is the third leading cause of mortality among Japanese, and individuals aged ≥65 years have a particularly high rate of death from pneumonia.[1)] In the elderly, aspiration pneumonia is a particularly common form of pneumonia because they are more susceptible to aspiration more easily due to deterioration of swallowing function. With the worldwide trend toward increasing mean ages of populations, the proportions of people with dysphagia because of deterioration of swallowing function are expected to increase in the future. Aged individuals commonly take a number of medications for a variety of aging-related illnesses. Even medications administered as treatment can sometimes cause aspiration pneumonia when they are accidentally swallowed. With this background, it is important to provide formulations that can be easily swallowed by patients with dysphagia.

Representative foods that can be easily swallowed include jellies, a variety of which have been sold for people with dysphagia. In addition, oral jellies have been listed as a type of dosage form in the Japanese Pharmacopeia, 16th Edition, enforced in 2011 in Japan and in the newer version. Jellies for oral administration are useful formulations that may be easily ingested by aged individuals and individuals with dysphagia caused by various diseases. However, oral jellies have various issues, including the need for more storage space because they are bulkier than solid formulations, such as tablets. These issues include transportation problems associated with the heavy weight of the amount of jelly prescribed for a substantial period (*e.g.*, the weight for a month is 2700 g if a jelly of 30 g weight is administered three times a day) and the need to be individually packed because of the concerns regarding microbial control and separation of water (oozing of water from jelly), which makes fine dosage adjustments difficult. Furthermore, in the industrial manufacturing process of conventional oral jellies, the active components, a polymeric gel base, and appropriate additives are converted into a homogeneous sol at high temperature, and the sol then is loaded into an airtight container followed by cooling to mold the jelly into an appropriate shape.[2)] Therefore, active components vulnerable to water and/or heat cannot be formulated into conventional oral jellies.

We have conducted research to develop novel jellies for oral administration that can solve these problems. In the present study, we developed a novel powder formulation that does not contain water when stored and becomes a jelly for oral administration after addition of water at the time of administration. We call such a formulation a dry jelly. According to the notation of the Japanese Pharmacopeia, dry jellies are described in "preparations for jellies." The aim of development of the dry jelly was to retain the advantages of ease of swallowing while eliminating the many issues of conventional oral jellies. The name dry jelly is based on its analogy to the widely known dry syrup formulations (preparations for syrups defined in the Japanese Pharmacopeia to form a syrup after the addition of water) because our developed dosage form becomes a jelly for oral administration immediately after the addition of water. To our knowledge, there have been no other reports on oral jellies as pharmaceutical preparations that can be gelated at the time of use by addition only of water to a dry powder.

Components that may be used as gel bases for dry jellies include, for example, pectin, sodium alginate, carrageenan, xanthan gum, carob bean gum, guar gum, and tara gum.[3)] Pectin has also been used as an additive to medications. Recently, there have been many reported results on the use of pectin, such as those from studies on a drug delivery system using microsized calcium pectin gel beads containing the active component[4–6)] and those from a study in which pectin

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was used as a base for a microneedle.[7] As these examples indicate, there are many practical applications of and research reports on the use of pectin as a pharmaceutical additive. Additionally, pectin is recognized as a safe ingredient[8] and has long been used as a food. Therefore, we mainly investigated the use of pectin as a suitable gel substrate in the dry jelly. In contrast to the objectives of the studies referenced above, the objective of the present study was to develop a dry jelly with physical properties that allowed easy swallowing, as characteristics of jellies for oral administration, and of homogeneous gelation with a certain degree of softness similar to that of a dessert jelly.

The main structure of pectin is polygalacturonic acid, which is composed of galacturonic acid residues linearly linked to each other through $\alpha(1\rightarrow4)$ bonds, and the carboxyl groups of galacturonic acid units may be methyl-esterified, amidated, or acetylated. The structure also contains neutral sugars, such as galactose and arabinose, in addition to galacturonic acid.[8] Generally, pectin is classified into high-methoxyl (HM) pectin, in which the degree of methoxylation (DM) is $\geq$50%, and low-methoxyl (LM) pectin, in which DM is $<$50%. DM affects the properties of pectin.[8] HM pectin can gelate at lower pHs (pH approximately $\leq$3.6) when a cosolvent (soluble solid content) is present (generally sucrose at a concentration of $\geq$55%).[9] LM pectin can gelate in the presence of divalent metal ions, such as calcium ion, by forming junction zones that are constituted by the binding of carboxyl groups in molecules with calcium ions. This junction zone is explained by an "egg box" model.[10,11] It is thought that LM pectin with a lower DM may have stronger gelation properties because it makes more junction zones. In addition, the physical properties of pectin are affected not only by DM but also by the pattern of methoxylation of the galacturonic acid backbone ["(absolute) degree of blockiness"].[12] In addition to these characteristics, pectin does not dissolve in water under suitable gelation conditions, similar to other gel bases.[11] In most cases, the pectin jelly can gelate after being heated to dissolve and then cooling down.[11] Moreover, pectin may gelate only under appropriate conditions, including the gelation temperature, pectin concentration, pH, soluble solid content, and calcium ions concentration.[11] Therefore, to use pectin as the gel base for oral jellies that may be prepared at the time of use, it is necessary to determine the factors related to control of the gelation conditions.

Considering the points of view mentioned above, we investigated gelation of a novel dosage form (dry jelly) containing pectin as its main gel base that does not contain water when stored and becomes a jelly for oral administration after the addition of water immediately before administration. First, in a simple system containing pectin, glucono-δ-lactone (GDL), dibasic calcium phosphate hydrate (DCPH), and sucrose, we studied the effect of the type of pectin and amounts of all constituents on gelation.

In this article, the state in which the aforementioned four components are mixed is described as a "dry jelly," and the state in which water has been added to the dry jelly to gelate is described as a "gel." Because water added to a dry jelly is a factor that greatly affects gelation, the influence of the hardness and quantity of the water was studied. In addition, drugs were added, and the effects on the physical properties of the gel and dissolution properties of the drugs were examined.

Experimental

Materials Pectin used for the preparation of dry jellies was manufactured by CP Kelco (GA, U.S.A.) and provided through SANSHO (Osaka, Japan). GDL (FUSO CHEMICAL, Osaka, Japan), DCPH and anhydrous sodium hydrogen phosphate (Taihei Chemical Industrial, Osaka, Japan), sucrose (Hiranoya, Osaka, Japan), and citric acid hydrate (SATUMA KAKO, Kagoshima, Japan) were used.

Acetaminophen (Sigma-Aldrich, MO, U.S.A.) was used as a test drug added to the dry jellies. The water used for the tests was purified by using an Elix Water Purification System (Integral-3; Merck, Darmstadt, Germany) unless otherwise specified. The natural waters used to study the effect of hardness of waters on dry jellies were purchased at supermarkets; their hardness values were obtained from the product labels.

All other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Methods

Measurement of DM of Pectin

DM was measured according to the Food Chemical Codex.[13] The following test solutions were prepared in advance.

Solution A: Purified water was added to aqueous hydrochloric acid 5 mL and 2-propanol 60 mL, and then the total volume was adjusted to 100 mL.

Solution B: One gram of phenolphthalein was dissolved in 95% ethanol, and the total volume was adjusted to 100 mL.

Five grams of pectin and 100 mL of solution A were placed in a beaker, and the mixture was stirred for 10 min. The mixture was transferred to a sintered-glass filter and washed with 15 mL of solution A six times. Then, the mixture was washed with 60% (v/v) 2-propanol until hydrochloric acid was completely removed. The time at which white turbidity was no longer observed after addition of one drop of silver nitrate solution to the washed solution was considered as the time when the complete removal of hydrochloric acid was accomplished. Finally, the mixture was washed with 2-propanol 20 mL. Then, the mixture was dried under 105°C for 2.5 h, cooled, and dried in a desiccator overnight. The resulting product 500 mg was transferred to a 250-mL Erlenmeyer flask and moistened with 2 mL of 2-propanol, 100 mL of carbon-dioxide-free water was added, and the mixture was stirred until the product was completely dissolved in water. Then, five drops of solution B was added, and the mixture was titrated with 0.1-mol/L sodium hydroxide solution. The volume of sodium hydroxide solution added was V1. Furthermore, 20 mL of a 0.5-mol/L sodium hydroxide solution was added, and then the mixture was vigorously shaken and left to settle for 15 min. Next, 20 mL of 0.5-mol/L hydrochloric acid was added to the solution, the mixture was shaken until the pink color disappeared, three drops of solution B were added, and the solution was titrated with 0.1-mol/L sodium hydroxide solution. The volume of sodium hydroxide solution added was set as V2.

DM was calculated according to the following equation.

$$\mathrm{DM}(\%) = \mathrm{V2} / (\mathrm{V1} + \mathrm{V2}) \times 100$$

Basic Formulation of the Dry Jelly and Its Conversion Procedure to Gel

The basic formulation in the present study is shown in Table 1. The components shown in Table 1, excluding water, were placed in a polyethylene bag and shaken well, and the dry

jelly was prepared. Water was added to the dry jelly so that the weight ratio of dry jelly and water was 1 : 3, and the mixture was immediately stirred for 30 s. Then, the mixture was allowed to stand still to gelate. A batch of the dry jelly was prepared in a sufficient quantity for multiple gel preparations. Gelation was evaluated by using the portion of the dry jelly batch required for a single gel preparation. The compounding ratio of the basic formulation was partially changed according to the study content; however, the weight ratio of dry jelly and water was always maintained at 1 : 3, and the amount of dry jelly was adjusted by the compound ratio of sucrose.

Preparation of Dry Jelly and Evaluation of the Physical Properties of Resulting Gels

In the Japanese Pharmacopeia, jellies for oral administration are defined as non-flowable gelatinous preparations having a certain shape and size; if the dosage form flows, it falls into the category of liquids and solutions for oral administration.[2] Using this definition, we visually confirmed whether the resulting gel lacked a flow property and qualitatively evaluated the presence or absence of gelation. For the gels in which gelation appeared to occur, their stress–strain curves were measured by using a creep meter to evaluate the gel's physical properties quantitatively. The breaking stress in the gel's physical properties was expressed by gel hardness, and the breaking strain was expressed as the strain.

The creep meter used for the measurements of the gel's physical properties was a RHEONER II RE2-3305C (Yamaden, Tokyo, Japan). A load cell of 200 N was used, and the magnification rate of the amplifier was set at 10 times. A cylindrical plunger compressed the gel by 90% of its thickness at a speed of 1 mm/s at 25°C.

Gelation in a Container and Evaluation of Hardness (Evaluation of Fluidity and Measurement of Gel Hardness)

Gels in containers were evaluated. A container (aperture diameter, 40 mm; height, 30 mm; volume 26 mL; polypropylene container with a taper; Sarstedt, Nümbrecht, Germany) was used. Two grams of the dry jelly was weighed and placed in the container, 6 g of water was added, and the mixture was immediately stirred with a muddler for 30 s. The reaction time was measured from the time when the stirring was over. After a specific time (1, 5, 15, 30 min), the fluidity of the content was evaluated. The container was turned upside down, and if the content dripped from the container, it was evaluated as no gelation took place; if it did not drip, it was evaluated as gelation. For gels that did not drip, the physical properties were measured by using the creep meter and a cylindrical plunger with a diameter of 16 mm and a height of 25 mm, and the stress–strain curve was plotted. In cases in which a distinct peak was obtained in the stress–strain curve, the maximum stress value was recorded as the gel hardness value. When the peak was ambiguous, the maximum stress values indicated continuously were recorded as the gel hardness value.

Table 1. Basic Formulation

Components	Contents (% (w/w))
Pectin (degree of methoxylation: DM39.8%)	1.5
Dibasic calcium phosphate hydrate (DCPH)	2.0
Glucono-δ-lactone (GDL)	2.0
Sucrose	19.5
Purified water	75.0

Gel Taken out of the Container and Evaluation of Gel Properties (Evaluation of Formability and Measurement of Gel Hardness and Strain)

The gel was taken out of the container and evaluated. One gram of the dry jelly was weighed and placed in a container (diameter, 26.3 mm; height, 22.0 mm; cylindrical polypropylene container; AS ONE Corporation, Osaka, Japan) and 3 g of water was added; the mixture was immediately stirred with a muddler for 30 s. The reaction time was measured from the end of stirring; after 30 min, the content was carefully taken out so that the contents were not destroyed, and the gel formability was evaluated. The content was determined to be a gel by its ability to keep the container shape. For the contents that retained the container shape (gels), their physical properties were measured by using a creep meter and a cylindrical plunger with a diameter of 40 mm and a height of 8 mm, and the stress–strain curve was plotted. In this measurement, a single distinct peak was obtained in the stress–strain curve; therefore, the gel hardness and strain of the peak apex were investigated as the gel hardness and strain, respectively.

Evaluation of pH as a Contributing Factor for Gelation

A 2-g portion of the dry jelly was weighed and placed in a container (diameter, 24 mm; height, 50 mm; volume, 14 mL; glass container; AS ONE Corporation), 6 g of water was added, the lid was put on, and the mixture was shaken for 30 s. The reaction time was measured from the end of shaking, and the pH of the mixture was measured every 30 s by using a pH meter (main unit, D-52; Electrode, 6252-10D; HORIBA, Kyoto, Japan).

Effect of Dry Jelly Containing Drug on Gelation and Evaluation of Drug Dissolution

An aluminum composite film was cut into a 4-cm square. The four sides were heat sealed, and a container having a space of a rectangular parallelepiped (1-cm sides, 1.5-cm height) was prepared (Fig. 1(a)). Acetaminophen of approximately 37 mg and dry jelly of approximately 0.212 g, which was prepared with a reduced amount of sucrose to accommodate the acetaminophen, were weighed and placed in this container, and the mixture was mixed sufficiently to yield a dry jelly containing drug. Then, water of approximately 0.75 g was added, and the mixture was stirred for 30 s. The reaction time was measured from the end of stirring, and after 5 and 30 min, the molded film was torn so as not to damage the gel; then the gel was removed. The gel was in the shape of a cube with approximately 1-cm sides (Fig. 1(b)) and was placed in a dissolution medium. Drug dissolution was measured after the gel was placed in the solution. The dissolution test was performed according to the paddle method described in the General Tests, Processes, and Apparatus in the Japanese Pharmacopeia[14] and used the NTR-6100 dissolution test device (Toyama Sangyo., Osaka, Japan) and water as the dissolution medium. The samplings were performed at 5, 10, 15, 20, 30, 40, 60, and 180 min. The absorbance at 242 nm of the sample solutions was measured by using a Hitachi U-2900 spectrophotometer (Hitachi High-Technologies, Tokyo, Japan) to calculate the dissolution rate.

Results and Discussion

Concept of Dry Jelly Formulation LM pectin forms a gel by reacting with calcium ions.[10,11] In addition, pectin

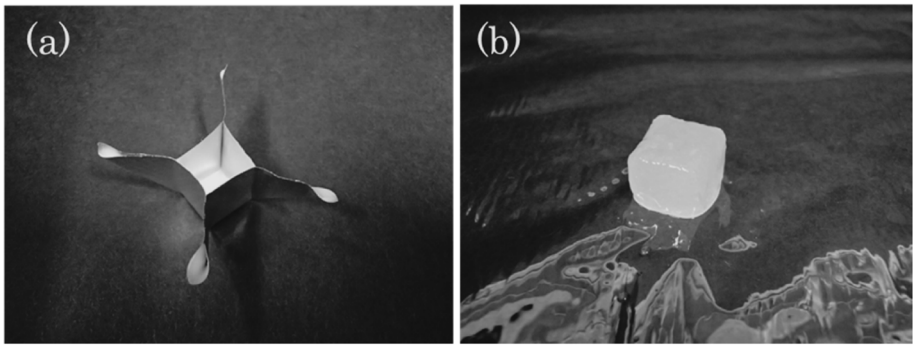

Fig. 1. An Aluminum Composite Film and Configuration of Gel Used for Dissolution Tests

(a) Mold formed by film; (b) Cuboid gel.

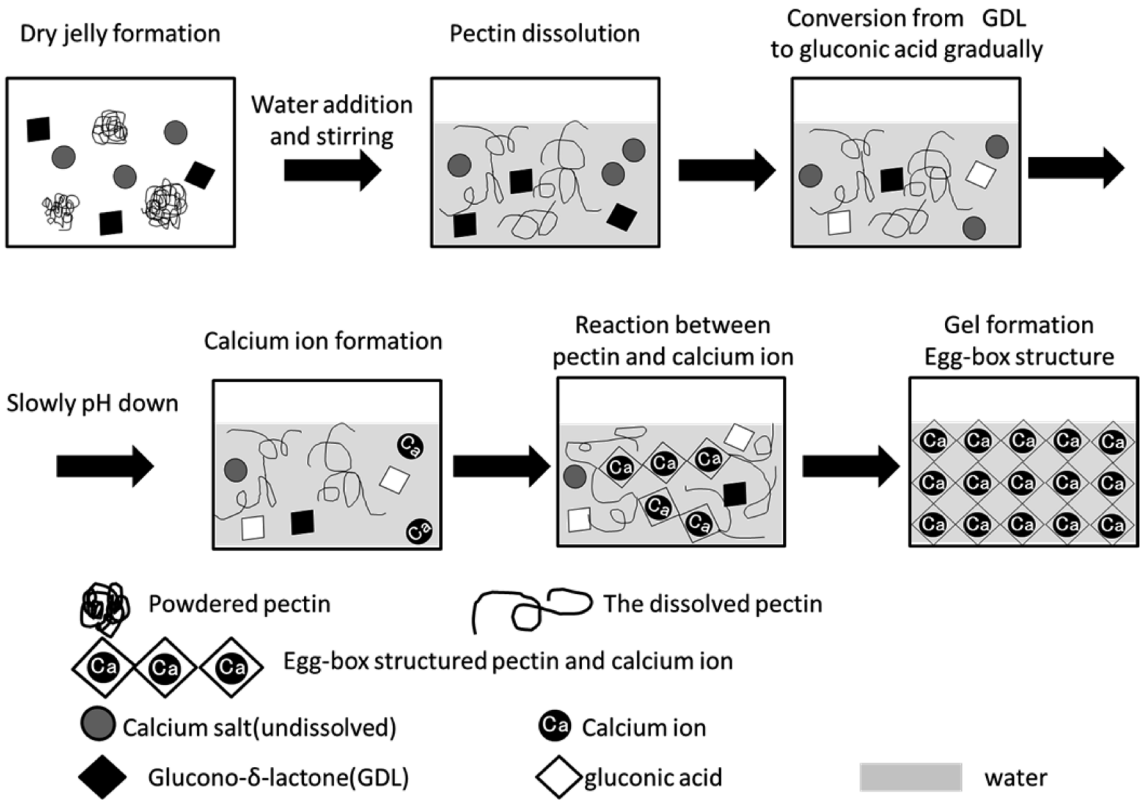

Fig. 2. Conceptual Diagram of Gel Formation from Dry Jelly

Shown is the ideal process to form a gel from the dry jelly by adding water. The egg box structure of pectin and the dissolution of pectin shown in various schematic diagrams are described in many papers.[8,10,16] The schematic here was constructed in a similar way.

does not dissolve in water under suitable gelation conditions, behavior that is similar to that of other general gel bases.[11] In fact, when adding a solution containing a large amount of acidic aqueous solution or calcium ions to pectin, it can be easily confirmed that the pectin is not dissolved. Additionally, since the viscosity is increased if the pectin is dissolved in water, it can be easily determined that it was dissolved. In this study, we examined the basic formulation, in which a gel is easily formed by adding only water before use, by observing the above-mentioned appearance changes. The pectin used for the study was a product for food production (dessert jellies) in industrial use, and its DM was measured 39.8%. The dosage form has to be prepared at the time of use so it has to gelate within a short period. We examined the possible formulations to form gels within 1 min after reacting. To form a gel quickly, the formulation concentration of pectin was fixed at 1.5% (w/w) slightly more than the manufacturer's recommendation. We decided to disperse with an appropriate amount of sucrose to dissolve the pectin efficiently. Because it becomes difficult to dissolve and lumps are formed when the pectin is mixed directly in water, the well-known method of adding sucrose to prevent lump formation was used.[11] Pectin reacts with calcium ions, there is a characteristic that does not dissolve in water calcium ions are contained in a large amount.[11] As a calcium ion source is added to the DCPH, the acidic component is added to the GDL.

DCPH hardly dissolves in water but does dissolve in the presence of acid.[15] Therefore, DCPH does not interfere with the solubility of pectin even if mixed with pectin. The solution also contains GDL that dissolves in water, and hydrolyzes into

gluconate, then leads the decrease in pH gradually, and after that the DCPH dissolves efficiently and releases its calcium ions when the pectin is dissolved.[15] The concept of dry jelly gelation by water addition is shown in Fig. 2.

Candidates for acidic components other than GDL were preliminarily examined and eliminated, because citric acid changed the pH drastically in a small amount, and fumaric acid, succinic acid, and adipic acid did not have enough solubility to dissolve DCPH and form a gel in 1 min. The DCPH and GDL concentrations were adjusted so that a gel could be formed within 1 min. Table 1 shows the basic formulation that was selected.

In addition, sucrose not only helped dissolve the pectin, but it was also used as an excipient to maintain the use of a constant amount of dry jelly. This made it easy to handle the dry jelly, and water could always be mixed at a rate of 1 : 3. When the ratio of sucrose to water was changed while holding constant concentration of pectin and DCPH and GDL of the basic formulation, gelation occurred without adding sucrose, and when sucrose was added in the range of 14.5–22.0% (w/w), it did not affect the physical properties. Subsequent studies in which the sucrose was increased or decreased and the amount of added water was constant, the effect of the sucrose amount on the gel properties was only slightly and was almost negligible.

The target dry jelly dosage form was based on these components. The dosage form has to be prepared at the time of use so it has to gelate within a short period. We examined a formulation that formed a gel within 1 min after adding water.

Table 2. Degree of Methoxylation (DM) of Pectin (Mean Value, $n=3$)

Pectin	DM (%)
A	15.2
B	19.1
C	35.3
D	39.8
E	38.7
F	46.6
G	41.6
H	60.9

In addition, we investigated a formulation that maintained the state of the gel for 30 min, assuming that it take time to take the gel after adding water.

Effects of Dry Gel Components on Gel Properties

Effect of DM of Pectin on Gelation and Gel Properties

The results of the measurements of DMs of eight different commercially available pectin preparations are shown in Table 2. One type out of eight was an HM pectin with a DM of 60.9%, and the other seven types were LM pectins. According to the basic formulation shown in Table 1, powdery mixtures were prepared from these eight types of pectin for which DMs were measured. Water was added to the dry jelly, the mixture was immediately stirred, allowed to stand still, and then gelation was evaluated. Gelation occurred for LM pectins with DMs ranging from 15.2–46.6%, but gelation did not occur for HM pectin with a DM of 60.9%. The physical properties of the gel in a container were measured, and the results are shown in Fig. 3. In pectins with DMs ranging from 19.1% to 46.6%, those with lower DMs tended to have higher gel hardness values. However, the pectin with a DM of 15.2% showed extremely low gel hardness despite the low DM. The gel hardness of jellies taken out of the containers showed tendencies similar to those shown in Fig. 3. DM at 15.2% gave a strain of 34%, DM at 19.1% gave a strain of 45%, and DMs at 35.3–46.6% gave a strain of 50%. Since the LM pectin gel is formed by crosslinking between the carboxyl group and calcium ions of the two pectin chains, the gel-forming ability is known to increase because the DM of pectin is low.[8,17] Gelation of pectin is defined by the stoichiometric ratio R, as shown in the following equation:

$$R = 2[Ca^{2+}]/[COO^{-}]$$

When the ratio R is 0.5, theoretically, all calcium ions contribute to the egg box structure.[8] However, it has been reported that an excess of calcium ions causes excessively fast local gelation followed by phase separation phenomena, syneresis, or precipitation of the pectin, which leads to a decrease in gel hardness, and on the other hand, excessively high pectin concentration decreases in the gel hardness.[8,18,19] From these reports, it is considered that without an appropriate R condition, a homogeneous gel structure cannot be constructed be-

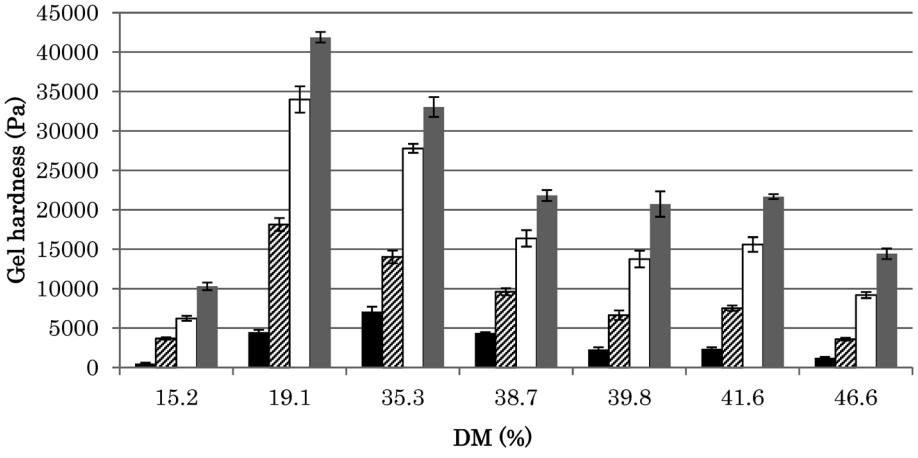

Fig. 3. Gel Hardness Values of Pectin Gels with Different Degrees of Methoxylation (DM) in a Container

Gels were prepared by adding water to dry jellies produced with pectin having different DMs, and the gel hardness values were measured over time. Values are the mean±standard deviation of five measurements. ■ means at 1 min, ▨ means at 5 min, □ means at 15 min, and ▩ means at 30 min.

Table 3. Formulations That Examine the Effects of Pectin Concentrations

Components	Concentration (% (w/w))							
	A	B	C	D	E	F	G	H
Pectin (degree of methoxylation: DM39.8%)	0.5	1.0	1.5	2.0	2.5	5.0	10.0	22.0
Dibasic calcium phosphate hydrate (DCPH)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Glucono-δ-lactone (GDL)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Sucrose	21.5	21.0	20.5	20.0	19.5	17.0	12.0	0.0
Purified water	75.0	75.0	75.0	75.0	75.0	75.0	75.0	75.0

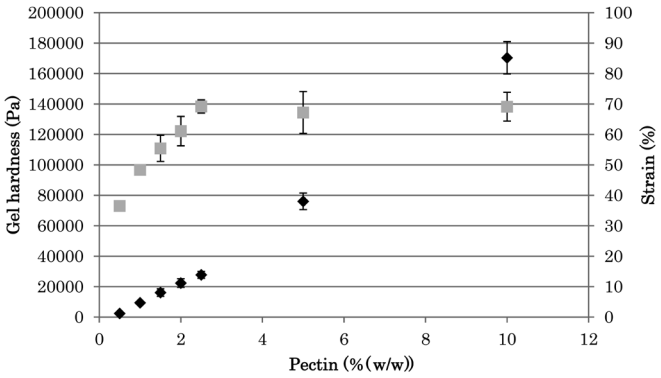

Fig. 4. Gel Hardness and Strain Values of Gels with Different Concentrations of Pectin Taken Out of a Container

Dry jellies were prepared with different concentrations of pectin and were converted to gels by adding water. The gel hardness and strain values were measured at 30 min after the gels were taken out of a container. ◆ mean gel hardness values, and ■ mean strain. Values are the mean±standard deviation of five measurements.

cause of local reactions between pectin and calcium ions. For a DM of 15.2%, it is considered that gel hardness and strain were lowered by the local reaction between the excess carboxyl group and calcium ions. If all the DCPH in dry jelly is dissolved, a local reaction between pectin and calcium ions is considered to occur because the R ratio is clearly >0.5, even if the DM is $\geq$19.1%. However, it is thought that the frequency of occurrence of local reaction decreased because the fewer carboxyl group reacted with calcium ions under the high DM condition so that a more homogeneous gel was formed. That was the reason why the gel hardness decreased, but the strain remained constant when the DM was higher. It is suggested that controlling the reaction of pectin and calcium ions is also important for dry jellies as well as the preparation of common pectin gels. We confirmed that in the range of DMs used in the present study, LM pectin was able to gelate within 1–30 min after the reaction.

Effect of Pectin Concentration on Gelation and Gel Properties

We evaluated the gelation with the formulation shown in Table 3. As a result, dry jellies containing 1–10% (w/w) of pectin were considered to undergo gelation between 1 and 30 min. Measurement of the physical properties of pectin showed that the gels in containers and gels taken out of the containers exhibited increasing gel hardness proportional to the pectin concentration over time. The gel hardness and strain of pectin taken out of a container are shown in Fig. 4. In Fig. 4, the strain increased proportional to the concentrations of pectin within the range of 0.5–2.5% (w/w); however, the strain corresponding to the pectin concentrations of 2.5–10% (w/w) were substantially constant at approximately 70%. The gel hardness refers to the force required for breaking the gel, and the strain indicates the percentage of deformation at the gel breaks. Therefore, gel hardness could increase infinitely, but the strain cannot logically be >100%. For this reason, the strain reached a limit at a pectin concentration of $\geq$2.5% (w/w).

In the fluidity evaluation of 0.5% (w/w) pectin, gelation did not occur after 1 min but did occur after 5 min. When the pectin content was 22% (w/w), the pectin absorbed water and formed a large lump; therefore, it was determined that no gelation occurred. The amount of dry jelly was adjusted by the amount of sucrose, and when the amount of pectin was increased, the amount of sucrose was decreased. Therefore, when the pectin content was 22% (w/w), the vast majority of the solid was pectin (Table 3-H). It is known that direct addition of water to pectin causes formation of a lump, which indicates that pectin is not soluble in water.[11] Sucrose is commonly added to pectin to prevent lump formation and to make pectin more soluble in water. Therefore, it is considered that when the pectin content is increased, increasing the sucrose content will prevent lump formation and allow gelation even at a pectin content of 22% (w/w). We confirmed that in the range of 1–10% (w/w) of pectin, gelation occurred within 1–30 min after the reaction.

Effect of GDL Concentration on Gelation and Gel Properties

The formulation rate of GDL of the basic formulation shown in Table 1 was changed to 0–5% (w/w), and the evaluation of gelation was performed. As a result, the range of GDL that caused gelation according to both the fluidity and formability evaluations was 0–3% (w/w). A GDL content of 5% (w/w) was determined to cause gelation by the fluidity evaluation but not by the formability evaluation.

Increasing concentrations of GDL led to decreasing pH when 0.25–5% (w/w) of GDL was added, and the pH tended to be more quickly lowered over time (the pH was 5.87–4.88 after 1 min, and 5.56–3.85 after 30 min). The results of the measurements of the physical properties of gels in containers are shown in Fig. 5. Comparison of various forms of the jellies having the same length of reaction time showed that for concentrations of GDL $\leq$2% (w/w), the gel hardness increased proportionally to the concentration; however, when the GDL concentration was $\geq$3% (w/w), the gel hardness tended to decrease as the concentration increased. Under the condition of higher GDL concentration, the pH decreased quickly, and then the dissolution of DCPH was promoted. The excessive calcium ions were reacted locally with pectin, and phase separation phenomena occurs. This was considered to be the cause of the decrease in gel hardness.

GDL was added with the aim of gradually lowering the pH

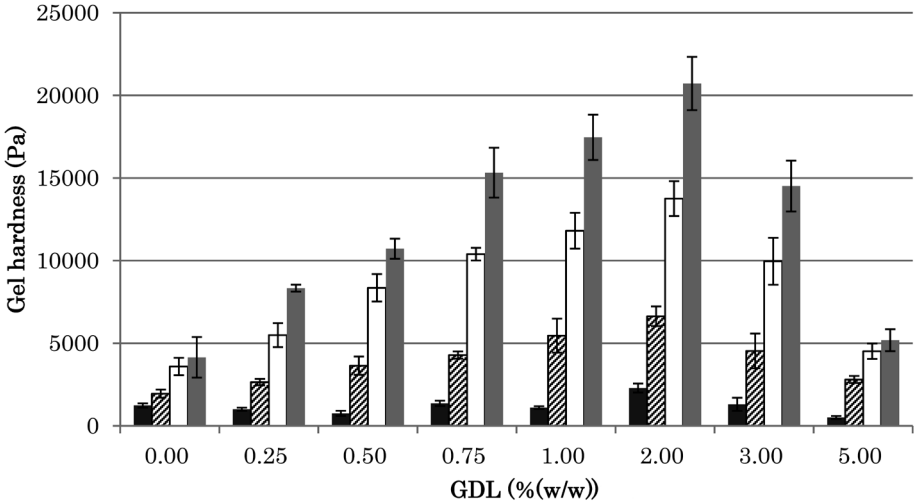

Fig. 5. Gel Hardness Value of Gels in a Container with Different Concentrations of Glucono-δ-lactone (GDL)

Dry jellies were prepared with different concentrations of GDL and converted to gels by adding water. The gel hardness values of the resulting gels were measured over time. Values are the mean±standard deviation of five measurements. ■ means at 1 min, ▨ means at 5 min, □ means at 15 min, and ▩ means at 30 min.

to dissolve DCPH; however, gelation took place even in the powder containing 0% (w/w) GDL. The pH of a gel containing 0% (w/w) GDL was 5.86 after 1 min, 5.92 after 5 min, 5.98 after 15 min, and 6.00 after 30 min. Even when GDL was not added, dissolution of pectin made the pH slightly acidic, so DCPH dissolved and the pH increased; thus, DCPH gradually became less soluble and slowed down the rate of pH change. When the GDL concentration was 0% (w/w), an increase in gel hardness was observed 1–15 min after the mixing, which suggested that the increase corresponded with the pH change. Compared with the change after 1–15 min, the change in gel hardness was very slow 15–30 min after the reaction. Therefore, it is considered that when GDL is not added to the mixture, the changes in the physical properties of gels are small, so the amount of GDL was effective for controlling the dissolution of DCPH to obtain gels with higher gel hardness. We confirmed that in the range of 0–3% (w/w) GDL concentration, pectin gelated within 1–30 min after the reaction.

Effect of DCPH Concentration on Gelation and Gel Properties

On the basis of the formulation in Table 3, the pectin concentration was fixed to 1.5% (w/w), the DCPH concentration was changed to 0.5–22.5% (w/w), and the evaluation of gelation was performed. The range of DCPH that was determined to cause gelation according to both the fluidity and formability evaluations was 1–8% (w/w).

According to the fluidity evaluation, gelation did not take place after 1 min but took place after 5 min when the DCPH concentration was 0.5% (w/w). When the GDL was hydrolyzed, the pH decreased, which led to dissolution of the DCPH. In the case above in which the concentration of DCPH was 0.5% (w/w), calcium ions were not sufficient, which is thought to explain why the gelation did not occur in 1 min.

The sample containing 22.5% (w/w) of DCPH was determined to gelate by the fluidity evaluation but not by the formability evaluation. The results of physical properties of the gel that gelated are shown in Fig. 6. A tendency for higher DCPH concentrations to cause lower gel hardness was observed. When the concentration of DCPH was higher, we considered that the excessive calcium ions were reacted locally with pectin, and then phase separation phenomena occurred; therefore, we considered that a decrease in gel hardness was observed. We confirmed that in the range of 1–3% (w/w) DCPH concentrations, pectin gelated within 1–30 min after the reaction.

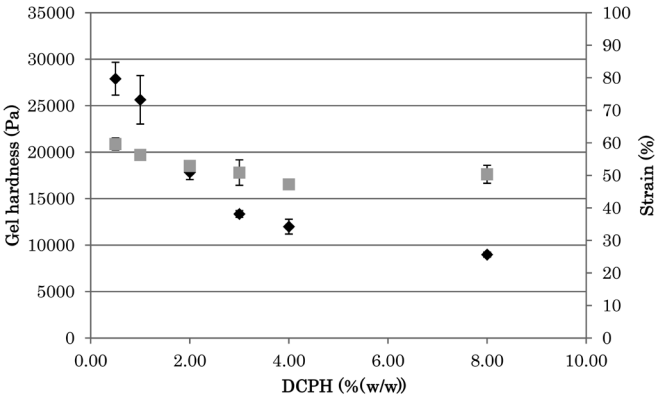

Fig. 6. Gel Hardness and Strain Values of Gels Taken Out of a Container with Different Concentrations of Dibasic Calcium Phosphate Hydrate (DCPH)

Dry jellies were prepared with different concentrations of DCPH, and water was added for gelation and shape formation. The gel hardness and strain values were measured at 30 min after the gels were taken out of a container. ◆ means stress values, and ▩ means strain. Values are the mean±standard deviation of five measurements.

To summarize the study results described above regarding the potential for gelation of dry jelly using pectin as the gel base, we found that when LM pectin was used with sufficient concentrations of pectin and calcium to achieve the necessary hardness, gelation occurred under a relatively wide range of conditions. However, by adding an excess amount of calcium to accelerate the gelation, we found that the physical properties of the gel tended to continue to change widely. We found that the choice of DM of pectin and control of the solubility of calcium by GDL were important for control of the physical properties of the dry jelly using pectin as the gel base.

Control of Physical Properties of Dry Jelly-Derived Gel

Gel Hardness Stabilization of Dry Jelly-Derived Gel

In the section "Effects of Dry Gel Components on Gel Properties," the range of conditions under which gelation oc-

curred were described. In most of the experiments performed in the study, the gel hardness continued to change widely. For the practical use of dry jellies, it is considered that wide and continuous change of gel hardness may not be desired because it may affect ingestibility. In the section, "Effect of GDL Concentration on Gelation and Gel Properties," we confirmed that when GDL was not added to the formulation, a rapid change in gel hardness was suppressed. The basic formulation shown in Table 1 was the formulation with GDL. GDL was eliminated from the basic formulation shown in Table 1 and replaced with the same amount of sucrose. In this formulation, the gel hardness of gel in a container was measured ≤120 min. The values of gel hardness at 30, 60, and 120 min were 20720, 21694, and 22900 Pa (with GDL), and 2994, 3970, and 4585 Pa (without GDL), respectively. We confirmed that even in the formulation with GDL, the wide change in the physical properties was converted into gradual change at 30 min. The formulation without GDL showed a gradual change in the physical properties even 120 min after gelation and remained soft.

Effect of Metal Ions in Water Added to the Dry Jelly on Gelation Properties

It is known that pectin gelation is affected by a variety of ions.[20,21] For example, in this study, gelation occurred in the formulation in which GDL was eliminated from the basic formulation shown in Table 1 after addition of citric acid solution at pH >3; however, in cases in which citric acid solution with pH of 2.9 and buffer solution with pH of >3 were added or in which sodium chloride was added, no gelation occurred. The causes may be that pectin does not dissolve to give a solution with low pH, and the gelation ability of pectin was weakened by the presence of sodium.[11] Purified water was used for the experiments thus far described; however, in the practical application of dry jellies, it is presumed that patients would use tap water, which may contain a variety of ions, to prepare gels from dry jellies. Therefore, we tested commercially available waters with different hardness values to see if they could induce gelation of dry jellies prepared by using two different formulations: one with GDL and one without GDL, as described in section "Gel Hardness Stabilization of Dry Jelly-Derived Gel." The hardness values of the waters used in this study were approximately 30, 304, and 1468 mg/L, respectively. The results are shown in Table 4 with the data obtained with purified water used in the earlier part of this study for which the hardness was approximately 0 mg/L. The highest hardness of water, 1468 mg/L, did not allow gelation of the formulation, regardless of the presence or absence of GDL. Water having approximate hardness values of 30 and 304 mg/L allowed gelation, regardless of the presence or absence of GDL. When GDL was present in the formulation, the gel hardness tended to be decreased after gelation in water with high hardness. In the formulation without GDL, the change in gel hardness was small, and the gel hardness tended to be somewhat high for a water hardness of 30 mg/L. Because extremely high hardness prevented gelation, the use of soft water was preferable; however, the formulation without GDL gelated in water with a hardness of 304 mg/L without affecting the gel hardness value significantly.

Effect of Amount of Water Added to Dry Jelly on Gelation Properties

We investigated the effect of concentrations of respective components in section "Effects of Dry Gel Components on Gel Properties." In the practical application of dry jellies, it is presumed that patients may have difficulty in measuring the amount of water accurately. Therefore, as described in section "Gel Hardness Stabilization of Dry Jelly-Derived Gel," the basic formulation with GDL and the formulation without GDL were mixed with the standard amount of water, half the standard amount, and two times the standard amount to observe the effects on the gels. The gel hardness values were measured and expressed as relative values to that of the standard one to which the standard amount of water was added, and the value was taken as 100%. The gel hardness of the formulations with or without GDL were 97 and 314% using the half amount of water and 46 and 23% using twice the amount of water, respectively. The gel became soft with a large amount of water and became hard with a small amount of water. The formation could gelate even when the amount of water was not accurate, and the gel hardness could be adjusted according to the preference of the user.

Evaluation of Physical and Drug Dissolution Properties of the Gel Prepared from Drug-Containing Dry Jelly

Physical Properties

The basic formulation shown in Table 1 was slightly modified to incorporate acetaminophen in dry jelly. Specifically, the amount of sucrose was reduced to accommodate 300 mg of acetaminophen in 2 g of dry jelly; the resulting preparation is referred to as the drug-containing dry jelly. Evaluation showed that gelation occurred after addition of water, and the addition of acetaminophen created no problem in fluidity or formability. The physical properties of the gel were measured and compared with those of the gels studied earlier that did not contain the active ingredient (placebo). As shown in Fig. 7, the gel hardness of the formulation with acetaminophen tended to be somewhat lower than that of the placebo when measured with the gels in containers. However, in the evaluation of gels taken out of containers, when the test of equal variance for two samples was applied to the gel hardness and strain of the placebo and acetaminophen formulation, no significant difference was found. It is considered that the acetaminophen-containing gel became slightly softer because the sucrose content was decreased by the amount of acetaminophen. However, the difference was not of significance, and the formulation contain-

Table 4. Effect of Metal Ions in Water Added to Dry Jellies on Gel Formation

	Purified water	Commercial item		
	0 mg/L	30 mg/L	304 mg/L	1468 mg/L
With GDL	20820±1613	18808±2392	8245±1462	No gel formation
Without GDL	2994±209	3899±734	3093±215	No gel formation

Dry jellies with and without GDL were prepared with purified water or commercially available waters of different hardness. The gel hardness values of gels in a container were measured at 30 min after the reaction. Values are the mean±standard deviation of five measurements.

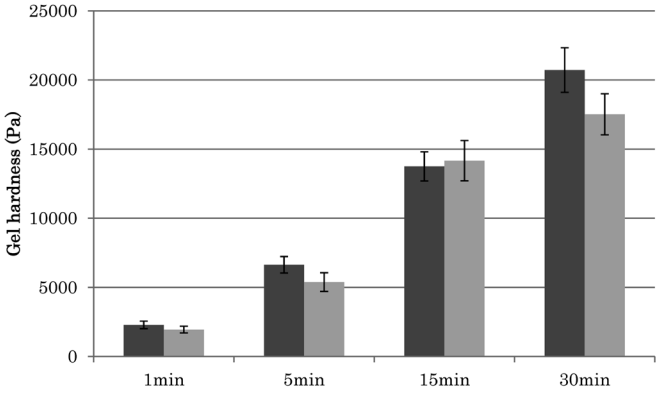

Fig. 7. Gel Hardness Values of Gel with Acetaminophen and Placebo

Dry jellies were prepared with and without acetaminophen; water was added to initiate gelation, and the gel hardness values of gels in a container were measured over time. ■ means for placebo, and ▒ means for acetaminophen. Values are the mean±standard deviation of five measurements.

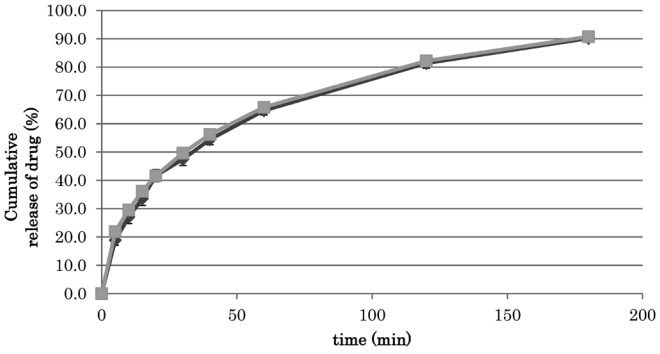

Fig. 8. Dissolution Properties of Acetaminophen in Gel at 5 and 30 min after the Reaction

Dry jellies were prepared with acetaminophen; water was added to initiate gelation, and the gel hardness values of gels in a container were measured over time. ◆ means of the dissolution test results at 5 min after the reaction, and ▒ means at 30 min. Values are the mean±standard deviation of three measurements.

ing acetaminophen as the active ingredient gelated similarly to that of the placebo without any problem.

Drug Dissolution Properties

The gel hardness values of gel containing acetaminophen of which the physical properties were measured in section "Physical Properties" were 5381 and 17518 Pa at 5 and 30 min, respectively, after the preparation. A 1-cm cube was made with a gel that showed widely different gel hardness values depending on the reaction time, and the dissolution test was performed. Similar dissolution curves were obtained from gels that had different gel hardness values (Fig. 8). We reported that there was no change in dissolution in the agar gels with different gel hardnesses, including acetaminophen. The reason for this is that if the gel structure is not dense even though the gel hardness is different, it is easy for acetaminophen molecules to pass through the structure of the gel and not to control dissolution.[22] A study on the microstructure of pectin reported that it has large pores of approximately 500 nm.[23] It is thought that there are large pores in dry jelly, which is the base of pectin. Therefore, it is thought that acetaminophen passed through the gel structure and released regardless of the gel hardness. Additionally, in a study that examined the transmission coefficients of each component after addition of bovine serum albumin, vitamin B12, and glucose to the agarose gel that changed the concentration, glucose was transmitted without being affected by the concentration of agarose.[24] The acetaminophen used in this study was a neutral compound with a lower molecular weight than that of glucose. Therefore, acetaminophen did not show interactions without relying on the pectin gel hardness, and it is thought that there was no difference in the dissolution. The dissolution properties did not change with changes in the physical properties after the reaction.

Conclusion

The goal of this study was to develop a novel dry jelly dosage form that did not contain water when stored and that could become a jelly for oral administration by the addition of water at the time of administration. Pectin was used as the gel base, and the influences of various related factors on gelation were studied. The concentration and DM of pectin and control of the dissolution of DCPH by GDL were important factors that affected the gelation of the dry jelly. When pectin is used as the gel base, metal ions contained in the water sometimes can inhibit gelation; however, when the optimum formulation was used, gelation occurred even with hard water (hardness of approximately 304 mg/L), and there were no changes in gel hardness. In addition, the desired gel hardness could be achieved by adjusting the amount of water. Furthermore, changes in the physical properties over time were observed after the addition of water; however, the changes did not affect the dissolution behavior of the test drug.

In this study, the physical properties and functionality of a formulation in which the raw materials were simply combined were evaluated. In the future, we will continue to study the conversion of dry jellies into granules and easily disintegrating tablets with improved physical properties that have good stability and are easily ingestible. We are considering different dry jellies using a variety of gel substrates other than pectin.

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Conflict of Interest Ohkura Pharmaceutical Co., Ltd. has a patent JP Patent 5414966. Yukari Kakino and Yoshihiro Hishikawa are employees of Ohkura Pharmaceutical Co., Ltd.

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