Development of Patient-Friendly Preparations: Preparation and Characterization of Allopurinol Gelatin Gel Containing Polyethylene(oxide)

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Summary: The pharmaceutical utility of allopurinol gel (APNgel), which consists of gelatin and polyethylene(oxide) (Alkox®), was investigated as a possible material for an oral dosage preparation for ease in handling and/or swallowing. The gel formation was studied as a function of the variety of Alkox® added and concentration of gelatin. The preferred composition of APNgel (Alkox®; gelatin % ratio) seemed to be that of 0.5:6.0, 0.5:8.0, 1.0:6.0 and 1.0:8.0. The gel strength and in vitro assessment of the adhesiveness of APNgels were evaluated using a creep meter. The gel strength of the APNgel was affected by the amount of Alkox® added. From the in vitro assessment of APNgel adhesiveness, the adhesiveness increased as the concentration of Alkox® increased. The release of APN from the APNgel was completed within 100–150s. The results obtained in this study suggest that the jelly-like preparations containing Alkox® have utility as a new dosage form which adhere to the oral mucosa.

Keywords: allopurinol; gelatin; polyethylene (oxide); adhesiveness; gel strength; stomatitis

Introduction

In recent years, cancer chemotherapy has been developed remarkably, yet the appearance of various side effects induced by antineoplastic agents is unavoidable.1 Stomatitis is well-known as one of the undesirable side effects induced by high and/or multiple dose of cytotoxic drugs such as 5-fluorouracil(5-FU). Stomatitis causes pain in the oral cavity, impaired swallowing or loss of appetite, and finally, lowering of the quality of life (QOL) of patients. With respect to stomatitis, it has been reported that cryotherapy using an “ice ball” or a “mouthwash” containing povidone iodine is available as a method for preventing the import of the antineoplastic agent into the oral mucosa or the removal of the free radicals induced in the oral cavity.2,3 Clark et al. demonstrated that allopurinol (APN) mouthwash diminished the mucositis resulting from 5-FU administration in colorectal cancer.3 Dozono et al. also reported that allopurinol mouthwash showed a marked effect on stomatitis induced by chemotherapy.4 APN mouthwash is usually made up of 1 mg/mL in 3% methylcellulose, 1% carboxymethylcellulose sodium or 0.1–0.2% sodium polyacrylate which act as a suspension or viscosity-increasing agents. In practice, however, it is difficult for patients to

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measure the exact volume of the mouthwash each time they are to gargle.

We have reported that jelly-like preparations are available as an oral dosage form for the elderly because of their ease of handling and swallowing. These jelly-like preparations have been prepared with various materials such as sodium caseinate, silk fibroin, and glycerogelatin.

In the present work, we attempted to prepare a jelly-like preparation of allopurinol gel (APNgel) consisting of gelatin and polyethylene(oxide). Gelatin has been used as a texture modifier and a gelling agent in the food industry. On the other hand, polyethylene(oxide) is obtained by ring-opening polymerization of ethyleneoxide which has high viscosity and spinnability. If the APNgel melts gradually in the mouth, the elongation of the adhesion time of APN onto the oral mucosa can be expected. The aim of this study was to investigate the effect of the mixing ratio of gelatin and polyethylene(oxide) on the physicochemical properties of the APNgel in vitro and its utility as a new oral dosage form and agent for reducing stomatitis.

Experimental

Materials

Allopurinol (APN) was purchased from Sigma (St. Louis, MO, U.S.A.). Gelatin (Type No.G-0442P, gel strength 167 Bloom, pH 5.7, water content 11.0%) and Alkox® (Alkox E-30; MW,300,000–500,000) were generously supplied by Nitta Gelatin Co. (Osaka, Japan) and Higuchi Inc. (Tokyo, Japan), respectively.

Preparation of APNgel

A fixed weight (0.5 g) of APN crystals, various weights (1–40 g) of gelatin and various weights of Alkox® (0.125–15 g) were dissolved in 500mL of distilled water at 70°C. The mixture was transferred to a 5.0-mL container and stored at 4°C.

Confirmation of gelation

A fixed volume (5.0 mL) of solution was placed in a 10-mL flat-bottomed cylindrical vial (diameter: 21 mm). The vial was tilted at definite time intervals, and the gelation of APNgel was defined as the time at which the solution formed a gel just strong enough to retain its shape in position.

Rheological measurements

Rheological measurements were carried out using a creep meter (Yamaden model 33005, Tokyo, Japan). A cylindrically cut APNgel (diameter: 20 mm, height: 16 mm) was placed in contact with a plunger, and the thickness of the APNgel was measured. The APNgel was loaded at the rate of 1 mm/s, and the stress force and strain were measured at the moment the APNgel broke.

Melting point measurement of APNgel

The melting point measurement of APNgel was carried out according to a previous study. A Teflon bar (length: 8 mm) was placed in the bottom of a screw-cap test tube, and the test solution was transferred into the tube. After gelation, the test tube was allowed to stand upside down in a water bath and was heated at a rate of 0.5°C/min. The temperature of the sample was measured using a digital thermometer (DFT - 600, Shinko Co. Ltd., Tokyo, Japan). The melting point was defined as the temperature at which the Teflon bar fell from the bottom of the screw-cap test tube during heating.

In vitro assessment of the adhesiveness of APNgel

The apparatus and brief principles are schematically shown in Fig. 1. Adhesive mea-
Fig. 1. Schematic View of the Measuring Method for the Assessment of Adhesiveness of APNgel

Measurements were carried out using a creep meter (Yamden model 33005, Tokyo, Japan). A fixed volume (5.0-mL) of APNgel (diameter: 20 mm, height: 16 mm) was placed on the sample holder, and the surface of a Teflon plunger (diameter: 20 mm) and the surface of the APNgel were allowed to come into contact. The top of the Teflon plunger was then immersed to a 1-mm depth. After that, the Teflon plunger was pulled up at the rate of 1 mm/s, and the adhesive force and displacement were measured when the plunger was completely separated from the surface of the APNgel.

Release study mimicking chewing action

In order to evaluate the dissolution behavior during chewing action in the mouth, we attempted the “paddle-bead method” to produce a mechanical impact force during dissolution as previously reported.\(^7\,10\) Polystyrene beads (diameter: 6.5 mm, Wako Pure Chemical Industries., Ltd., Osaka, Japan) were added to the dissolution test medium and the paddle rotation speed was set at 25 rpm; the number of beads used was 2,500. A 250-mL portion of distilled water (37°C) was used as the dissolution medium. A definite weight of cylindrical APNgel was inserted into the dissolution medium, and the released APN was quantitatively analyzed. A 5.0-mL portion of the fluids was removed at certain intervals, and the volume was kept constant by adding the same amount of dissolution medium at the same temperature.

Disintegration test of APNgel

The disintegration test of APNgel was performed according to JP XIII. Distilled water was used as the test fluid.

Syneresis study of APNgel

A known weight (2.0 g) of APNgel was stored in a desiccator at 25°C (65%RH). The weight change in the APNgel was measured at definite intervals. The amount of water
Table I. Gelation Behavior of the Gelatin Gel Containing Various Amounts of Alkox<sup>a</sup>

<table>
<thead>
<tr>
<th>Conc. of gelatin (%)</th>
<th>Conc. of Alkox&lt;sup&gt;a&lt;/sup&gt; (%)</th>
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<tr>
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<td>0.5</td>
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<td>2.0</td>
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<td>4.0</td>
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<td>6.0</td>
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<td>8.0</td>
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+.. Gelation was recognized; −, Gelation was not recognized; ±, In spite of the gelation being recognized, it was impossible to handle.

Results and Discussion

1. Gelation of gelatin gel containing various amounts of Alkox<sup>a</sup>

Table I shows the gelation behavior of the gelatin gel containing various amounts of Alkox<sup>a</sup>. When the concentration of Alkox<sup>a</sup> was adjusted to 0.5%, gelation was recognized in all gelatin concentrations used in this study, whereas in the case when the concentration of Alkox<sup>a</sup> was 4.0%, gelation was not observed. In the cases where the concentrations of Alkox<sup>a</sup> and gelatin (Alkox<sup>a</sup>-Gel) were (0.5–2.0), (0.5–4.0), (1.0–4.0) and (2.0–4.0), gelation was recognized in a flat-bottom glass vial. However, the samples were shaped like rice porridge and seemed to be unfavorable for handling. From a practical point of view, the preferential compositions of APNgel (Alkox<sup>a</sup>, gelatin % ratio) seemed to be 0.5:6.0, 0.5:8.0, 1.0:6.0 and 1.0:8.0, respectively. Furthermore, the precipitation of APN crystals in APNgel was not observed by the naked eye throughout this study.

2. Rheological study of APNgel

In order to evaluate the texture of various food hydrocolloids, rheological measurements have been carried out. In the case of this study, because the purpose of preparing the APNgel was that allopurinol dissolved from the APNgel would be retained in the oral cavity in a similar manner to that of APNmouthwash, the APNgel must melt or breakdown when administered. Figure 2 illustrates the stress-strain curves of gelatin gel and APNgel prepared with various Alkox<sup>a</sup> and gelatin contents. In the stress-strain curve, the slope of the initial portion indicates the initial modulus of elasticity of the APNgel; the smaller the slope of the initial portion, the more easily the APNgel deforms. That is, APNgel which shows a small breaking point and slope has suitable fragility. In this study, the breaking point of each gel, which indicates the firmness of the APNgel and the rupture of the APNgel taking place at this point, is shown by the arrows. In each stress-strain curve, because the yield points coincided with the breaking point, this behavior was confirmed to be brittle fracture. The load at the breaking point of the APNgel was smaller than that of gelatin jelly; however, a marked change in the breaking point with the addition of Alkox<sup>a</sup> was not observed. This was explained by presuming the existence of closely networked gelatin molecules in gelatin gels. On the other hand, with the addition of Alkox<sup>a</sup>, the APNgels showed lower breaking points. In this study, the reason for the decrease in the breaking point on adding Alkox<sup>a</sup> is not fully understood, but it might be related to the increase in viscosity among the APNgels. The gel breaking strain indicates the fragility of the material. In all APNgel samples, the gel breaking strain ranged from 70 to 79%. These values are similar to those of the gelatin jellies normally used for commercial jelly. This indicates that APNgel has both suitable fragility for easier swallowing and facility in disintegrating in the mouth or the esophagus. Furthermore, APNgel melted in the range from 34.0 to 40.2°C as listed in

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Table I: Gelation Behavior of the Gelatin Gel Containing Various Amounts of Alkox<sup>a</sup>

<table>
<thead>
<tr>
<th>Conc. of Alkox&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
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<tr>
<td>2.0</td>
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<td>4.0</td>
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<td>6.0</td>
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<td>8.0</td>
<td>+</td>
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</table>

+.. Gelation was recognized; −, Gelation was not recognized; ±, In spite of the gelation being recognized, it was impossible to handle.
Fig. 2 Stress-Strain Curves of APNgel
a: 6.0% gelatin gel, ■: APNgel comprised of 6.0% gelatin and 0.5% Alkox®, ●: APNgel comprised 6.0% gelatin and 1.0% Alkox®, ○: b: 8.0% gelatin gel, □; APNgel comprised 8.0% gelatin and 0.5% Alkox®, ▲; APNgel comprised 8.0% gelatin and 1.0% Alkox®, △.

| TABLE II. Breaking Stress and Adhesion Properties of APNgel Prepared with Various Amounts of Gelatin and Alkox® |
|---------------------------------------------------------------|-------|-------|-------|-------|-------|-------|
| Conc. of gelatin (%) | 0     | 0.5   | 1.0   | 0     | 0.5   | 1.0   |
| Conc. of Alkox® (%)  |       |       |       | 6.0   | 6.0   | 8.0   |
| Breaking stress (× 10⁴ N/m²) | 7.3±0.9 | 3.7±0.7* | 4.1±1.2* | 8.1±1.5 | 7.1±0.3 | 6.1±1.0 |
| Adhesion force (J/m²) | 30.2±3.5 | 38.0±5.6 | 63.0±4.5*** | 24.2±0.2 | 43.1±2.8 | 54.6±8.0* |
| Spinnability (%)     | 6.4±0.2 | 6.9±0.2* | 8.5±0.1*** | 5.5±0.6 | 6.2±0.4 | 7.0±0.2* |
| Melting point (°C)   | 29.0   | 34.0   | 36.5   | 29.5   | 35.4   | 40.2   |
| Disintegration time (s) | 49.0±6.0 | 63.0±4.2 | 59.0±1.0 | 51.3±4.5 | 51.0±1.0 | 78.0±2.0** |

* *, **, ***: Significantly different from control at p < 0.05, p < 0.01 and p < 0.005, respectively. Data are expressed as means ± SD where n = 3.

Table II, so it can be stored at room temperature (in JP XIII: 1–30°C)

3. In vitro assessment of the adhesiveness of APNgel

Figure 3 shows the portion of the load-displacement curves of APNgel, corresponding to the part “A2” shown in Fig. 1. The negative value of the load indicates the tension of the APNgel, and the area of the load-displacement curve indicates the adhesive energy between the surface of the plunger and that of the APNgel; the larger the area of the load-displacement curve, the higher the adhesion energy of the APNgel. At the same time, displacement indicates the spinnability of the APNgel; the longer the displacement, the larger the spinnability of the APNgel. As listed in Table II, the adhesion energy and displacement increased with the increase in amount of Alkox® added. As a whole, both adhesion energy and spinnability of the APNgel samples containing 6.0% gelatin were higher than that containing 8.0% gelatin. This seemed to be because the breaking stress of the APNgel containing 8.0% gelatin was more gelled and could retain its shape. From these results, when taking into account the adhesion of an APNgel to the oral mucosa, the APNgel consisting of 6.0% gelatin and 1.0% Alkox® seems to be favorable.
Fig. 3. Stress-Displacement Curves of APNgel
a: 6.0% gelatin gel, ■; APNgel comprised of 6.0% gelatin and 0.5% Alkox®, ●;
APNgel comprised of 6.0% gelatin and 1.0% Alkox®, ○. b: 8.0% gelatin gel, □;
APNgel comprised of 8.0% gelatin and 0.5% Alkox®, ▲; APNgel comprised of 8.0% gelatin and 1.0% Alkox®, △.

Fig. 4. Release Profiles of APN from APNgel Containing Various Amounts of Gelatin and Alkox®
Percent ratio of Alkox® and gelatin: ● (0.5 : 6.0), ○ (1.0 : 6.0), ▲ (0.5 : 8.0), △ (1.0 : 8.0).

4. Releasing study mimicking the chewing action
In practical use, the APNgel seems to be swallowed after melting or chewing. We attempted to evaluate the release profile of APN during melting or chewing by a "paddle-bead method." Taking into account the fact that the APNgel melted in the human mouth, the disintegration time of the APNgel samples seemed to be an indication of the release of APN. The disintegration times of the APNgel samples are listed in Table II. During the disintegration test, the APNgel was assumed to undergo dissolution rather
5. Syneresis of APNgel

It has been reported that gelatin gel can lose its own water. Watanabe et al. demonstrated that, even in the case of a 20% gelatin gel, the loss of water due to syneresis occurred after 10 days storage under 59%RH.\(^8\) In this study, in order to evaluate the stability of the APNgel during storage, syneresis of the APNgel during storage at room temperature (65%RH) was investigated. Figure 5 shows the syneresis profiles of APNgel samples stored in a desiccator at 25°C (65%RH). The APNgel gelled by 6.0% gelatin exhibited a higher syneresis than that with 8.0% gelatin. The reason for this seemed to be attributed to a difference in the extent of gelation between gelatin molecules; that is, at the same volume of APNgel, the larger the concentration of gelatin, the more closely and tightly the gelatin gela,tes, so that syneresis might be suppressed. On the other hand, the syneresis of APNgel containing 1% Alkox\(^8\) is higher than that of 0.5%. This might be because the water molecules in the APNgel containing 1% Alkox\(^8\) might be more mobile than that with 0.5% Alkox\(^8\). As for the many factors which influence the syneresis of APNgel, the interaction between water molecules and gelatin molecules and/or Alkox\(^8\) might be involved. However, further details on these phenomena can be revealed and are the subject of a future study.

In conclusion, APNgel consisting of gelatin and Alkox\(^8\) can be prepared. The addition
of Alkox® resulted in increased APNgel adhesiveness with increasing amounts of Alkox®. It was suggested that Alkox® be utilized as an adhesion agent to the oral mucosa. Based on the confirmation of gelation, however, when taking into account the adhesion of APNgel to the oral mucosa, the APNgel consisting of 6.0% gelatin and 1.0% Alkox® seems to be favorable. Though further detailed investigations of in vivo applications (including a sensory test), the subject of the administration of APN to patients, and the evaluation of practical therapeutic effects remain, the results obtained in this study suggest that the jelly-like preparations containing Alkox® have utility as a new dosage form that adheres to the oral mucosa.

References