Factors Affecting Prednisolone Release from Hydrogels Prepared with Water-Soluble Dietary Fibers, Xanthan and Locust Bean Gums¹⁾

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The release behavior of prednisolone from hydrogels prepared with xanthan and locust bean gums was investigated. Newly developed equipment was employed in order to increase the gum concentration in the hydrogels. The apparent release rate of prednisolone from the hydrogels decreased with increasing gum concentration, suggesting that the diffusion of drug molecules was mainly controlled by the density of the three-dimensional network structure in the matrix. The effect of additives such as glycerin and sucrose on the release behavior of prednisolone was also investigated in detail. Drug release was significantly lowered by the addition of these compounds to these hydrogels. A linear relation was observed between the apparent release rate of prednisolone and the microscopic viscosity of the hydrogels. These results indicated that the drug release could be controlled not only by the density of the network structure but also by the microscopic viscosity of the hydrogels.

Keywords hydrogel; xanthan gum; locust bean gum; release rate; prednisolone; three-dimensional network; swelling; microscopic viscosity; glycerin; sucrose

Hydrogels prepared with various polymer materials have been recently reported to be applicable as a device for obtaining sustained or controlled release of drugs in the pharmaceutical field.²⁻⁶⁾ In a previous paper,⁷⁾ we reported the drug release behaviors from hydrogels which were prepared with water-soluble dietary fibers, xanthan and locust bean gums. The application of these naturally occurring polysaccharides in the design of pharmaceutical preparations is considered to be useful since these compounds have high biocompatibility and biological safety.^{8,9)} However, the control of drug release from the hydrogels prepared with xanthan and locust bean gums was not sufficient because of the difficulty in enhancing the gum concentration in the hydrogels by the conventional preparation method. In this paper, we employed newly developed equipment in order to increase the gum concentration in the hydrogels. The release behavior of prednisolone from the hydrogels was studied for various gum concentrations. Furthermore, the effect of additives such as glycerin and sucrose was investigated, considering the microscopic viscosity in the drug diffusion route.¹⁰⁾

Experimental

Materials Xanthan and locust bean gums were generously supplied by Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). The weight-average molecular weight of xanthan gum was 2 million and the viscosity of a 0.5% aqueous solution was 500 cP at 25 °C. The number-average molecular weight of locust bean gum was 300—310 thousand and the viscosity of a 0.5% aqueous solution was 200 cP at 25 °C. Prednisolone was purchased

from Sigma Chemical Co. (St. Louis, U.S.A.). 4-Hydroxy-2,2,6,6-tetramethylpiperidino-1-oxy (4-hydroxy-TEMPO) was purchased from Eastman Kodak Company (New York, U.S.A.). All other chemicals used were of reagent grade.

Preparation of Hydrogels Newly developed equipment for preparing hydrogels is schematically represented in Fig. 1. Xanthan and locust bean gums were sieved and mixed in a mortar in the weight ratio of 1:1. Separately, prednisolone was dissolved in ethanol (10 mg/ml). A proper quantity of the mixed powders and 0.5 ml of the drug solution were loaded into syringe A in Fig. 1. Separately, an appropriate amount of the second disintegration test fluid (Japan Pharmacopeia XI) was poured into syringe B. After the two syringes were connected with a syringe-connector, the fluid in syringe B was then introduced into syringe A by pushing the piston rod of syringe B. After that, the contents in syringe A were injected into syringe B in the same manner. This process was repeated more than 10 times in order to disperse the gums and prednisolone in the fluid. After the contents were collected in syringe B, the tip of syringe B was sealed tightly, and then heated for 1 h at 90 °C to dissolve the gums in the fluid. Finally, the syringe was stored at 6 °C for 24 h. The obtained hydrogels (26 mm length and 15 mm diameter) were stored at 6 °C until use in the release study. In the case of the hydrogels containing glycerin or sucrose, these compounds were previously dissolved in the second fluid, and the preparation was performed by the same previously described manner.

Release Test A Toyama Sangyo (Osaka, Japan) NTR-VS type dissolution tester (rotating basket method) was used at a rotating speed of 50 rpm with 400 ml of the second disintegration test fluid at 37 °C. Samples (5 ml) were withdrawn at appropriate intervals through a Fine filter F (Ishikawa Seisakusho Co., Ltd., Tokyo, Japan) and immediately replaced with an equal volume of the test medium. Samples were analyzed spectrophotometrically at 247 nm for prednisolone using a JASCO Ubest-30 spectrophotometer (Japan Spectroscopic Co., Ltd., Tokyo, Japan).

Swelling Test Hydrogels were immersed in a beaker containing 50 ml of the second disintegration test fluid at 37 °C. At appropriate intervals, the hydrogels were taken and weighed accurately after removing the residual water on the gel's surface with filter paper. Swellability of the hydrogels was defined as dividing the swelled weight at time *t* by the initial weight of the hydrogels.

Microscopic Viscosity Measurement Microscopic viscosity of the hydrogels was measured using an electron spin resonance (ESR) probing technique.¹⁰⁾ 4-Hydroxy-TEMPO was used as the probe. According to the method previously described, the hydrogels were prepared with water containing 4-hydroxy-TEMPO (1×10^{-4} M) in a glass tube for the ESR spectroscopy. The spectra of 4-hydroxy-TEMPO were recorded at 25 °C using a JEOL JES-FE2XG ESR spectrometer at 5 mW power level and 4 G modulation amplitude. The microscopic viscosity of the hydrogels was calculated from the ESR spectra. Details for the estimation of microscopic viscosity are described later in this paper.

Fig. 1. Schematic Representation of Disposable Syringe Equipment for Preparing Hydrogels

Results and Discussion

Effect of Gum Concentration on Drug Release from Hydrogels The release phenomena of prednisolone from the hydrogels prepared with xanthan and locust bean gums in the weight ratio of 1:1 were investigated considering the effect of total gum concentration. In a previous study,⁷⁾ we investigated the drug release behaviors from the hydrogels in which the gum concentration was 3% (w/v) as a maximum. However, the controllability of drug release from such hydrogels was not sufficient because of the difficulty of raising the gum concentration in the hydrogels. In this study, newly developed equipment was employed in order to increase the gum concentration and, thereby, the concentration was successfully enhanced up to 20% (w/v) as a maximum. Figure 2 shows the release phenomena of prednisolone from the hydrogels prepared with the various gum concentrations. The amount of released prednisolone from the hydrogels gradually decreased with increasing gum concentration, suggesting that the three-dimensional network structure in the hydrogels became dense as the gum concentration increased. To investigate the effect of gum concentration more precisely, the results were analyzed according to the following equation.

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

where M_t/M_∞ is the amount of prednisolone (%) released

Fig. 2. Effect of Gum Concentration on Release Behavior of Prednisolone from Hydrogels Prepared with Xanthan and Locust Bean Gums in Weight Ratio of 1:1

Gum concentration: $\blacktriangle$, 1%; $\square$, 5%; $\circ$, 10%; $\bullet$, 20%. Each point represents the mean of three experiments.

Fig. 3. Effect of Gum Concentration on Apparent Release Rate, K , and Release Order, n , of Prednisolone

Each point represents the mean of three experiments.

at time t (h), n is a diffusional exponent and K is the apparent release rate (%/h ^{n}). These diffusion parameters, K and n , are represented as a linear function of the gum concentration, as shown in Fig. 3. The K values decreased linearly with increasing gum concentration, clearly indicating the diffusion of prednisolone molecules was mainly controlled by the density of the three-dimensional network structure in the hydrogel matrix. On the other hand, the n values increased linearly from 0.537 to 0.618 with increasing gum concentration from 1 to 20% (w/v), suggesting that the mechanism of drug release from the hydrogels gradually changed from a Fickian type ($n=0.5$) to a non-Fickian type ($n>0.5$) diffusion as a function of the gum concentration.^{11,12)} In general, it has been observed that the diffusional exponent in the matrix type diffusion is gradually enlarged by the swelling of a matrix accompanying the chain relaxation mechanism.^{13,14)} The hydrogels prepared in this study became swellable as a function of time and the degree of swelling increased with increasing gum concentration (Fig. 4). This might be the main reason that a non-Fickian type diffusion was obtained in the hydrogels prepared with the higher concentration of gums.

Effect of Additives on Drug Release from Hydrogels The effects of glycerin or sucrose added to the hydrogels on the release behaviors of prednisolone are shown in Fig. 5. In both cases, the drug release was observed to decrease with increasing concentration of these additives. Armstrong

Fig. 4. Effect of Gum Concentration on Swelling of Hydrogels Prepared with Xanthan and Locust Bean Gums in Weight Ratio of 1:1

W_0 means the initial weight of hydrogels. W_t means the weight of hydrogels at time t . Gum concentration: $\blacktriangle$, 5%; $\circ$, 10%; $\blacksquare$, 20%. Each point represents the mean of three experiments.

Fig. 5. Effect of Glycerin (a) or Sucrose (b) Added on Release Behavior of Prednisolone from Hydrogels Prepared with Xanthan and Locust Bean Gums in Weight Ratio of 1:1 at 10% (w/v) of Gum Concentration

Concentration of additives: $\bullet$, 0%; $\triangle$, 30%; $\blacktriangle$, 50%; $\blacksquare$, 70%. Each point represents the mean of three experiments.

Fig. 6. ESR Spectra of 4-Hydroxy-TEMPO at 25°C in Distilled Water and in Hydrogels Containing Sucrose

(a) distilled water, (b) 30% (w/v) sucrose, (c) 50% (w/v) sucrose, (d) 70% (w/v) sucrose.

et al. have reported that the microscopic viscosity of the hydrogels plays an important role in determining the drug diffusion through the hydrogels.¹⁰⁾ Therefore, we tried to estimate the microscopic viscosity of the hydrogels under test according to the ESR probing technique.¹⁰⁾

When small molecules of nitroxide such as 4-hydroxy-TEMPO are moving rapidly and isotropically in a non-viscous medium, the ESR signal consists of three narrow and symmetrically spaced peaks of equal height. If the molecules of the spin probe are not freely tumbling, this may lead to a differential line broadening in the spectrum. Namely, the amplitude of the high-field line will be decreased with increasing the microscopic viscosity. Figure 6 shows the ESR spectra of 4-hydroxy-TEMPO as a spin probe in distilled water and in the hydrogels (10% (w/v) of the gum concentration) containing various amounts of sucrose. The amplitude of the peak at high field (the right peak) decreased gradually as a function of the amount of sucrose added, suggesting the increase in the microscopic viscosity of the hydrogels as increasing the sucrose concentration. Similar phenomena were observed in the case of glycerin.

The mobility of 4-hydroxy-TEMPO is assessed in terms of a rotational correlation time (τ) which is determined from the ESR triplet.

$$\tau = 6.5 \times 10^{-10} \cdot \Delta H_0 [(h_0/h_{-1})^{1/2} - 1] \quad (2)$$

where h_0 and h_{-1} are the amplitudes of the central and high field lines, respectively. ΔH_0 is the line width of the central peak in gauss. The coefficient of Eq. 2 gives the τ value in nanoseconds. The microscopic viscosity of the hydrogels (η_{gel}) were estimated from τ values (ns) of the hydrogels (τ_{gel}), as follows:

$$\eta_{\text{gel}} = \eta_{\text{H}_2\text{O}} \cdot \tau_{\text{gel}} / \tau_{\text{H}_2\text{O}} \quad (3)$$

where $\eta_{\text{H}_2\text{O}}$ is the viscosity of water (0.8904 cP at 25°C)

TABLE I. Rotational Correlation Time, τ , of 4-Hydroxy-TEMPO in Distilled Water and in Hydrogels, and Estimated Microscopic Viscosity

Solvent	Gum concentration (%(w/v))	τ (ns)	Microscopic viscosity (cP)
Water	0	0.031	0.890
Water	1	0.038	1.091
Water	5	0.034	0.977
Water	10	0.049	1.407
Water	20	0.054	1.551
Glycerin 10%	10	0.053	1.522
30%	10	0.078	2.240
50%	10	0.127	3.648
70%	10	0.202	5.802
Sucrose 10%	10	0.056	1.608
30%	10	0.091	2.614
50%	10	0.161	4.624
70%	10	0.320	9.191

Fig. 7. Relationship between Apparent Release Rate, K , and Microscopic Viscosity of Hydrogels Prepared under Various Conditions

a) Hydrogels were prepared with various gum concentrations. A numeral in the parentheses means the gum concentration. b) Hydrogels were prepared with 10% (w/v) gum concentration containing various amounts of glycerin. A numeral in the parentheses represents the concentration of glycerin. c) Hydrogels were prepared with 10% (w/v) gum concentration containing various amounts of sucrose. A numeral in the parentheses represents the concentration of sucrose. Each point represents the mean of three experiments.

and $\tau_{\text{H}_2\text{O}}$ is the rotational correlation time of 4-hydroxy-TEMPO in water. The results are listed in Table I. In the case of hydrogels without additives such as glycerin and sucrose, the change in their microscopic viscosities was very little despite a gum concentration change from 1 to 20% (w/v). In contrast, the microscopic viscosity estimated from hydrogels in which glycerin or sucrose were added was significantly increased with increasing concentration of these additives. The relationship between the apparent release rate, K , of prednisolone and the microscopic viscosity of the hydrogels is shown in Fig. 7. In the case of hydrogels without any additives, no relationship was observed between the K values and the microscopic viscosity (Fig. 7a). On the other hand, a linear and negative relation was clearly observed between the K values and the microscopic viscosity in the case of the hydrogels containing glycerin and sucrose (Figs. 7b and 7c). As a possible mechanism, it was considered that the additives such as glycerin and sucrose might increase the diffusion resistance by increasing the microscopic viscosity of the hydrogels.¹⁵⁾

In conclusion, drug release from the hydrogels prepared with water-soluble dietary fibers was considered to be controlled by the gum concentration and/or the additives

such as glycerin and sucrose. In future work, we intend to investigate the *in vivo* applicability of these hydrogels as a device for the controlled release of drugs.

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References and Notes

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