Characteristics of Drug Release from Gel Beads Formed by Hydrolysis of Alginic Acid into Guluronic Acid Blocks

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Alginic acid (Alg) is a natural anionic polysaccharide, which consists of α-L-guluronic acid (G) and β-L-mannuronic acid (M). G-G sequence-rich chain regions, known as G-blocks (GB), are important regions for gelation of Alg using divalent cations. In this study, calcium-induced GB gel beads were prepared, and drug release profiles and degradation properties of the GB gel beads were investigated in aqueous media. The GB gel beads swelled slightly in JP XVI 1st fluid (pH 1.2), and only slight release of sodium diclofenac (DF) from the GB gel beads was observed. Disintegration of the GB gel beads was not observed in the 1st fluid. On the other hand, the GB gel beads disintegrated in JP XVI 2nd fluid (pH 6.8), and the rate of disintegration depended on the concentration of calcium chloride used to prepare the GB gel beads. The DF release profiles of the GB gel beads in the 2nd fluid could be controlled by the concentration of CaCl₂ used to prepare the GB gel beads. The initial release profile of DF from GB gel beads was not consistent with the profile of disintegration. According to the Higuchi-plot of the percentage of drug content released against the square root of time, gel disintegration did not affect the release of DF from GB gel beads. It appears that a diffusion-type mechanism was responsible for DF release. We propose that the GB gel bead gel matrix is an effective medium by which to control the release of drug within the gastrointestinal tract.

Key words guluronic acid; gel bead; alginate hydrolysate; drug release profile

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a constant volume. The sample was mixed with 0.3 mL of 50 mM sodium phosphate buffer (pH 7.0) and centrifuged at 10,000 rpm for 5 min. The supernatant was filtered (Millex-HV, 0.45 μm; Merck Millipore, Billerica, MA, U.S.A.) and subjected to HPLC analysis. The amount of GB in the solution was determined by the method of Matsubara et al.,10 with some modifications. A 50-μL aliquot of the diluted sample was loaded onto a high-performance gel permeation column (Superdex 75 10/300 GL, 10 mm × 300 mm; GE Healthcare Bio-Sciences AB) and eluted with 50 mM sodium phosphate buffer (pH 7.0) as the mobile phase, using a flow rate of 0.5 mL/min (L-2130; Hitachi, Tokyo, Japan). The GB in the effluent from the column was detected at 210 nm using a UV spectrophotometer (L-2400; Hitachi).

**Drug Release Test** The release of DF from GB gel beads into JP XVI 1st fluid or JP XVI 2nd fluid was determined. Dried GB gel beads, corresponding to 0.1 g of hydrogel, were added to 20 mL of test medium maintained at 37°C in a glass vial. The vial was shaken at 250 rpm in a shaker incubator at 37°C. A 0.7-mL aliquot of the solution was removed periodically for analysis and replaced with 0.7 mL of fresh test medium (pre-warmed to 37°C) to maintain a constant volume. The sample was centrifuged at 10,000 rpm for 5 min, and a 0.6-mL aliquot of the supernatant was mixed with 0.6 mL of fresh test medium. The absorbance of each diluted sample was determined with a spectrophotometer (UV-1200; Shimadzu, Kyoto, Japan) at 275 nm. All tests were performed in triplicate.

**Results and Discussion**

**GB Formation** GB, which are sequences of G-G, were obtained by partial degradation with dilute HCl and separated by using the method of Haug et al.17 We previously described gel permeation chromatography of Alg and its hydrolysates. The molecular weight of GB was one-eighth that of Alg.16 The fluidity of the GB suspension influenced the formation of GB gel beads. In fact, spherical beads did not form when highly viscous solutions, such as 12% GB, were used. Spherical hydrogel beads formed immediately after a 10% GB suspension was added drop-wise into 0.02–2.0 M CaCl₂. The diameter of the dried GB gel beads is shown in Table 1. As the CaCl₂ concentration used to prepare the GB gel beads was increased from 0.2 to 2.0 M, the diameter of the dried GB gel beads increased. The GB gel beads incorporated drug into their gel matrix. DF was detected within the dried GB gel beads at 84.3% of its theoretical yield. Although a 10% suspension of GB also resulted in formation of spherical hydrogel beads in 0.01 M CaCl₂, the GB gel beads were very soft, rendering them unable to be washed with distilled water.

**Disintegration of the GB Gel Beads** When a dried GB gel bead was soaked in aqueous solution, it either swelled, disintegrated, or both. The GB gel beads swelled slightly in the 1st fluid (pH 1.2), but did not disintegrate within a 3-h incubation period. The total amount of GB released from the GB gel beads into the 1st medium is shown in Fig. 1A. Release of GB from the GB gel beads was not observed by HPLC analysis after 3 h, and this result was consistent with visual observations.

When a GB gel bead prepared with 0.02–2.0 M CaCl₂ was soaked in the 2nd fluid (pH 6.8), the gel was visibly eroded. As shown in Fig. 1B, after 3 h, 73.7% of the components of the gel matrix were released from the GB gel beads prepared with 0.2 M CaCl₂. The GB release rates decreased with exposure to increasing concentrations of CaCl₂ during preparation.

**Drug Release from the GB Gel Beads** Drug could be readily incorporated within the matrices of the GB gel beads. In all cases, the drug loading capacity of the GB gel beads exceeded 77%, as shown in Table 1. As the CaCl₂ concentration used to prepare the GB gel beads was increased from 0.02 to 2.0 M, the drug loading capacity of the GB gel beads increased. It was reported that the DF loading capacity of Alg gel beads prepared with 0.2 M CaCl₂ is 67.2%.18 The GB gel beads prepared with 0.2 M CaCl₂ concentration incorporated 84.3% of DF into their gel matrix. Thus, the ability of GB gel beads to incorporate drugs is superior to that of Alg gel beads.

The release profiles of DF from the GB gel beads were observed, and the release rates were not affected by the CaCl₂ concentrations used to prepare the GB gel beads. The release profiles of drug from the GB gel beads in the 1st fluid were similar to that of Alg gel beads.13

Release of DF from the GB gel beads increased dramatically when disintegration of the gel matrices occurred in the

### Table 1. Diameter and Drug Loading Capacity of GB Gel Beads

<table>
<thead>
<tr>
<th>CaCl₂ concentration (M)</th>
<th>Diameter (mm)</th>
<th>Loading capacity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>0.93±0.09</td>
<td>77.9±3.1</td>
</tr>
<tr>
<td>0.2</td>
<td>0.94±0.08</td>
<td>84.3±3.2</td>
</tr>
<tr>
<td>2.0</td>
<td>1.23±0.12</td>
<td>94.6±4.7</td>
</tr>
</tbody>
</table>

a) Diameter data represent the mean±S.D. (n=20). b) Loading capacity data represent the mean±S.D. (n=3).
The concentrations of CaCl₂ used to prepare the GB gel beads were as follows: open circles, 0.02 m; closed triangles, 0.2 m; and closed squares, 2.0 m. (A) Release of GB in the 1st fluid. (B) Release of GB in the 2nd fluid. Data represent the mean ± S.D. (n=3).

The concentrations of CaCl₂ used to prepare the GB gel beads increased from 0.02 to 2.0 m. However, the release rate constants in the 2nd fluid could be controlled by the concentration of CaCl₂. The release rates of DF from the GB gel beads decreased as the concentration of CaCl₂ used to prepare the GB gel beads increased from 0.02 to 2.0 m. However, the initial release profile of DF from GB gel beads prepared with 2.0 m CaCl₂ was not consistent with the initial release profile of GB from the GB gel beads prepared with 2.0 m CaCl₂. In order to understand mechanism of drug release, the profiles of release of DF from GB gel beads were used to plot the percentage release of drug against the square root of time (Fig. 3). The profiles of release of DF from GB gel beads were linear on the Higuchi plot in the range of about 5% to 90%, and the slope decreased as the concentrations of CaCl₂ used to prepare the GB gel beads increased. The release rate constants in the 2nd fluid from the beads prepared with 0.02 m, 0.2 m, and 2.0 m CaCl₂ were 11.7%/min 1/2 (r²=0.989), 8.9%/min 1/2 (r²=0.994), and 3.1%/min 1/2 (r²=0.997), respectively. According to these observations, the release of DF from GB gel beads was not affected by gel disintegration, and it appears that the mechanism of DF release was diffusion type. The controlled release of a water-soluble drug, DF, from the GB gel beads could be controlled by the concentration of CaCl₂ used to prepare the GB gel beads.

The concentrations of CaCl₂ used to prepare the GB gel beads were as follows: open circles, 0.02 m; closed triangles, 0.2 m; and closed squares, 2.0 m. Data represent the mean ± S.D. (n=3). There was a significant difference between the release rate constant of the GB gel beads prepared using 0.02 m CaCl₂ and that of the GB gel beads prepared using 0.2 m CaCl₂ (p<0.05). Furthermore, there was a significant difference between the release rate constant of the GB gel beads prepared using 0.2 m CaCl₂ and that of the GB gel beads prepared using 2.0 m CaCl₂ (p<0.05).

Fig. 2. Effect of Calcium Concentration on DF Release from GB Gel Beads in the 1st Fluid (pH 1.2) or the 2nd Fluid (pH 6.8)

Fig. 3. Plots of Release of DF from GB Gel Beads in the 2nd Fluid against the Square Root of Time

The concentrations of CaCl₂ used to prepare the GB gel beads were as follows: open circles, 0.02 m; closed triangles, 0.2 m; and closed squares, 2.0 m. Data represent the mean ± S.D. (n=3). There was a significant difference between the release rate constant of the GB gel beads prepared using 0.02 m CaCl₂ and that of the GB gel beads prepared using 0.2 m CaCl₂ (p<0.05). Furthermore, there was a significant difference between the release rate constant of the GB gel beads prepared using 0.2 m CaCl₂ and that of the GB gel beads prepared using 2.0 m CaCl₂ (p<0.05).

Conclusion
In this study, we prepared calcium-induced GB gel beads and investigated their drug release profiles. We demonstrate that the release of DF from the GB gel beads in the 1st fluid was slight and that release rates were not affected by the CaCl₂ concentrations used to prepare the GB gel beads. On the other hand, the DF release profiles of the GB gel beads in the 2nd fluid could be controlled by the concentration of CaCl₂ used to prepare the GB gel beads, although controlled release of a water-soluble drug, DF, from Alg gel beads in the 2nd fluid was difficult. The GB gel beads suppressed the release of DF in the 1st fluid and controlled the release of DF in the 2nd fluid. Therefore, the use of GB gel beads is feasible for controlled release in the small intestine of drugs that have the side effect of gastric ulcer. We propose that GB gel beads show promise with regard to the development of controlled release drug formulations.


References


