# Interaction of Calcium-Induced Alginate Gel Beads with Propranolol

Naoki SEGI, Toshihisa YOTSUYANAGI* and Ken IKEDA

*Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467, Japan.* *Received April 25, 1989*

**The loading capacity of calcium-induced alginate gel beads for propranolol, selected as a cationic model drug, was investigated with particular attention to the effects of excess $Ca^{2+}$ in the beads, pH and drug concentration in the bulk solution. The amount of the drug loaded in the excess $Ca^{2+}$-washed beads was higher than that in the corresponding unwashed beads. Decreasing the pH from 4 to 1 induced size contraction of the washed beads, for which changes of the fraction of ionized carboxyl groups of the polymer seemed to be responsible, and the $pK_a$ value was estimated to be 2.8. The drug loading increased in a sigmoid mode with increasing bulk drug concentration when loaded at pH 3—4. It is especially noteworthy that the dramatic increase of the drug loading was accompanied with abrupt contraction of the beads and transformation from a slightly translucent bead body to whitish beads in the vicinity of the inflexion point, and reached a saturated level that depended on the pH. Such physical appearance changes seemed to be due to the precipitation of the drug–polymer complex in the beads but it did not show crystalline nature. The loading capacity could be controlled by adjusting the pH of the medium, and the precipitated form of the drug possibly acts as a good reservoir for efficient drug release.**

**Keywords** alginate gel bead; loading capacity; propranolol; pH effect; size change

## Introduction

Alginic acid can spontaneously form a translucent gel in association with calcium ions, and the mechanism of gelation, for which guluronic acid blocks are mainly responsible, has been intensively investigated by circular dichroism (CD) and nuclear magnetic resonance studies.[1-5]

It seems possible that alginate gel beads can be applied as an orally-administered drug delivery vehicle. Because of the reswelling properties of the beads, which are susceptible to environmental pH, the following advantages may be envisaged: (1) acid-sensitive drugs, if incorporated in the beads, would be protected from gastric juice, (2) the reswelling process of xerogels in the intestine offers controlled-release drug delivery, (3) appropriately-sized particles of xerogels avoid local build-up of released drugs, (4) alginate is known to be nontoxic when taken orally.[6]

Also, alginic acid has ion-exchange properties because of its COOH groups. Ion binding properties of the polymer have been investigated from several different viewpoints.[7-9] The relative binding abilities of divalent cations to bring about the gelation of alginates have been investigated by several authors.[10-15] Also, interaction of alginate with univalent cations in solution has been investigated by CD and rheological measurements.[16]

Likewise, possible interaction between alginate gel and a drug could be a primary factor in the preparation of drug-incorporating gel beads, especially where the drug is cationic. The purpose of this paper is to describe the interaction between the alginate gel beads and propranolol, which is used as a model cationic drug ($pK_a$ 9.45), and the drug-loading capacity of the gel beads, in addition to their morphological changes.

## Experimental

**Materials** Na-alginate (Lot No. ARO1, Tokyo Kasei Kogyo, Tokyo) was used after dialysis against distilled water using Visking cellulose tubing (36/32) for 3d (three water replacements/d) followed by lyophilization. The mannuronate/guluronate ratio was 1.3.[6] Calcium chloride, dihydrate (special grade, Wako Pure Chem. Industries, Ltd., Osaka) was used. Propranolol hydrochloride (special grade, Aldrich Chemical Co.) was used. All other chemicals were of reagent grade.

**Preparation of Fully-Cured Alginate Gel Beads** Alginate gel beads were prepared by dropping Na-alginate solution (2, 3, 4, and 5% (w/v) in distilled water) into 0.1 M $CaCl_2$ solution, using a peristaltic pump (MP-3, Tokyo Rikakikai Co., Tokyo) with a polyethylene-tubing nozzle (0.50 mm i.d. and 0.80 mm o.d.). The pumping rate was 10 beads/min. The falling distance was 3.5 cm. The gel beads, which were allowed to stand in the $CaCl_2$ solution for more than 3d, were assumed to be fully cured.[6] The term fully-cured beads is used to describe the ones that were simply harvested from the $CaCl_2$ solution. The beads therefore contain calcium ions which are not associated with the gelation of alginate.

**Preparation of Washed Alginate Gel Beads** Fully-cured gel beads formed in 0.1 M $CaCl_2$ were washed five times for 5 h with freshly distilled water to remove excess calcium ions which are not associated with the gelation. The gel beads were allowed to stand in distilled water until use.

**Determination of the Size of Alginate Gel Beads** The size of beads was determined by taking a photomicrograph of each bead (×20) and measuring its diameter at three different positions. The average value was taken.

**Determination of the Amount of Water in Alginate Gel Beads** Gel beads were dried by heating in an oven at 110 °C for 3 h. The weight difference before and after drying was assumed to be the amount of water in the beads.

**Loading Propranolol into Gel Beads** Ten washed gel beads were placed in distilled water or buffer solutions (10 ml, pH 2.0, 2.5, KCl–HCl buffer; pH 3.0—4.0, acetate buffer) containing various concentrations (5—150 mM) of propranolol. Loading of fully-cured gel beads was done in the drug solution (35 mM) containing $CaCl_2$ (0.1 M). The gel beads were allowed to stand in the drug solution for 24 h. The temperature was 25 °C.

**Determination of Propranolol Loaded in Gel Beads** The drug-loaded gel beads were taken from the drug solution and disintegrated in 5 mM ethylendiaminetetra acetic acid (EDTA)–0.2 M phosphate buffer (pH 7.2) solution. After 24 h, the concentration of propranolol was determined spectrophotometrically at 289 nm.

## Results and Discussion

**Physical Dimensions of Fully-Cured Alginate Gel Beads and Washed Alginate Gel Beads** Table I shows the physical dimensions of fully-cured gel beads and washed gel beads. The washed beads seemed to be very slightly swollen compared with the fully-cured beads containing excess $Ca^{2+}$. It was reported previously that the physical appearance of the beads remained unchanged during washing and $Ca^{2+}$ was quantitatively and firmly associated with the polymer molecules at the ratio of $1.6 \times 10^{-3}$ mol/g of polymer.[6]

The weight difference of the dried fully-cured beads and the washed beads allowed us to calculate the content of excess $Ca^{2+}$ in the fully-cured beads, assuming that the

TABLE I. Physical Dimensions of Fully-Cured Gel Beads and Washed Gel Beads

| Concn.[a] (w/v%) | Radius[b] (cm) | Volume[c] ($\times 10^3$ cm$^3$) | Weight[b,c] (mg) | Water content (%) | Density (g/cm$^3$) |
|---|---|---|---|---|---|
| Fully-cured gel beads | | | | | |
| 2 | 0.113 | 6.04 | 6.20 | 94.7 | 1.03 |
| 3 | 0.121 | 7.36 | 7.60 | 93.9 | 1.03 |
| 4 | 0.123 | 7.86 | 8.17 | 92.7 | 1.04 |
| 5 | 0.127 | 8.53 | 8.91 | 92.4 | 1.05 |
| Washed gel beads | | | | | |
| 2 | 0.121 | 7.42 | 7.52 | 96.9 | 1.01 |
| 3 | 0.127 | 8.58 | 8.75 | 96.0 | 1.02 |
| 4 | 0.129 | 8.99 | 9.24 | 94.5 | 1.03 |
| 5 | 0.131 | 9.50 | 9.74 | 94.0 | 1.03 |

*a*) Initial alginate concentration. *b*) Average of 20 beads. *c*) Values per bead.

difference is solely due to $CaCl_2$ in the beads. For example, the weight difference of the 5% alginate beads is 0.093 mg/bead (Table I) which corresponds to $8.38 \times 10^{-7}$ mol/bead. Alternatively, the amount of the ions may be calculated, assuming that the beads were filled with 0.1 M $CaCl_2$ solution. The calcium content in the bead is given $8.23 \times 10^{-7}$ mol/bead because $0.1 \times 8.91 \times 0.924 \times 10^{-6} = 8.23 \times 10^{-7}$. These two independently estimated values agreed reasonably well with each other. It should be noted that there was little effect of the existing excess $Ca^{2+}$ on the size of the beads, in comparison with the propranolol loading into the beads described hereinafter.

**Interaction of Propranolol with Gel Beads** Table II shows the amounts of propranolol loaded into the fully-cured beads and the washed beads from the 35 mM drug solution, of which the pH was not adjusted but the ion strength was adjusted to 0.3 with NaCl. The loading capacities of the washed beads were 3—5 times higher than those of fully-cured gel beads. The washed beads were only slightly swollen (1.1—1.2 times larger), indicating that the increase of the amount of the drug loaded is not due to the volume increase but due to the removal from the beads of excess $Ca^{2+}$ which was not associated with the gelation. It is known that the gelation of alginic acid is predominantly caused by the interaction of $Ca^{2+}$ with guluronic acid blocks.[17,18] So, there should be extra COOH groups which mainly belong to mannuronic acid and heterogeneous blocks not involved or little involved in the gelation. The result suggests that cationic propranolol competes with $Ca^{2+}$ in the interaction with the COOH groups.

**Effect of pH on Propranolol Loading into Washed Gel Beads** The effect of pH on the loading capacity of propranolol was investigated using washed gel beads (initial alginate concentration of 4%) in the range of pH 1 to 4, above which the beads are gradually disintegrated.

Figure 1 shows the total amount of propranolol loaded in the beads as a function of the bulk concentration of the drug at various pHs. At pH 1—2, the amount of propranolol loaded in the beads was simply proportional to the bulk concentration of the drug. As the pH was increased, the relationship between the total amount of the drug loaded and the bulk concentration generally appeared sigmoid.

As suggested from the pH dependency of the loading capacity, it appears to be an important factor for the loading of cationic propranolol whether or not the COOH groups which are not associated with the gelation are dissociated. Because dramatic changes of the size contraction of the beads were observed depending on pH and on the bulk concentration of the drug, the volume of water in the beads was also measured (Fig. 2). The pH de-

TABLE II. The Amount of Propranolol Loaded in Alginate Gel Beads[a]

| Concn.[b] (w/v%) | Fully-cured gel bead[c] ($\times 10^7$ mol/bead) | Washed gel bead ($\times 10^7$ mol/bead) |
|---|---|---|
| 2 | 1.63 | 5.73 |
| 3 | 1.72 | 8.18 |
| 4 | 2.24 | 8.44 |
| 5 | 2.38 | 11.3 |

*a*) Propranolol solution (35 mM, pH not adjusted). *b*) Initial alginate concentration. *c*) $Ca^{2+}$ unwashed.

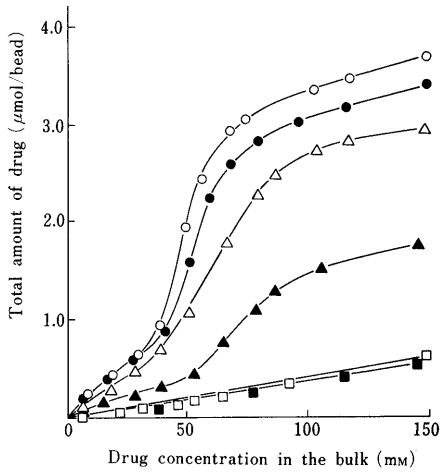


Fig. 1. Total Amount of Propranolol Loaded in Washed Gel Beads under Various pH Conditions

Beads were prepared with an initial alginate concentration of 4% (w/v) and washed with distilled water. Each plot reprsents an average value of 2—3 runs. ■, pH 1.0; □, pH 2.0; ▲, pH 2.5; △, pH 3.0; ●, pH 3.5; (○), pH 4.0.

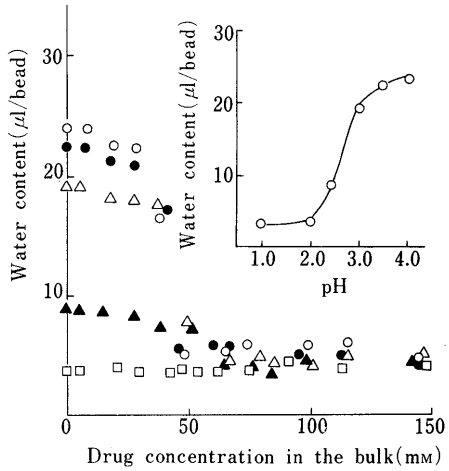


Fig. 2. Water Content Changes of Washed Gel Beads Caused by pH and Propranolol Loading

Beads were prepared with an initial alginate concentration of 4% (w/v) and washed with distilled water. Each plot represents an average value of 2—3 runs. Data at pH 1.0 are not shown since they are very similar to those at pH 2.0. Inset: Effect of pH on the water content per bead.

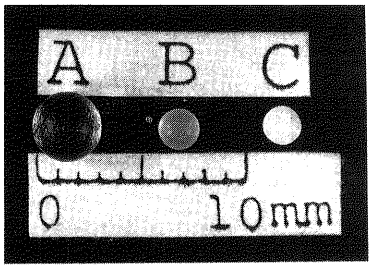


Fig. 3. Photographs of Size Change of Beads Caused by pH Changes and Propranolol Loading

Beads were prepared with an initial alginate concentration of 4% (w/v) and washed with distilled water. A, no drug loading at pH 4.0; B, pH 2.0; C, maximally drug-loaded beads ($3.7 \times 10^{-6}$ mol/bead) at pH 4.0.

pendency of the volume of the beads in which no drug was loaded revealed typical hydration behavior caused by the changes of the unionized and ionized fractions of the COOH groups, indicating that the apparent $pK_a$ value is about 2.8 (Fig. 2, inset). This value may not necessarily hold for uronic acid residues because of their highly concentrated state in the beads. It is, however, probable that the ionized groups repel each other at higher pHs and at the same time attract more water, resulting in expansion of the beads, and at pH 1—2, where the COOH groups remain unionized, the beads contract to a great extent due to loss of the electrostatic repelling force and reduction of the polymer dissolution.

The contraction of the beads was also caused by drug loading as effectively as by pH change, accompanied with physical appearance changes. The beads contracted slightly in size but remained unchanged with regard to their slightly translucent body at drug concentrations up to about 30 mM. As the drug concentration was further increased, sudden transformation to whitish beads, especially at higher pHs, was observed (Fig. 3). Such a transformation was not observed in the case of pH changes alone.

The contraction of the beads occurred in a very narrow range of drug concentration, finally attaining an almost identical level of water content regardless of the pH of the bulk drug solution. The minimum water content was the same as that in the case of simple equilibration in the acidic region (pH 1.0—2.0) without drug loading. These results indicate that there is a critical minimal amount of water required in the beads beyond which the beads can no longer lose water if the polymer concentration is kept constant. Since the pH was maintained constant in each drug loading study, the mechanism of contraction of the beads should be different from that caused by pH changes alone where changes of the fractions of unionized and ionized COOH groups were primarily responsible. The total amount of propranolol in the bead, $D_i$, may be expressed by

$$D_i = D_a + C_f V_g \quad (1)$$

where $D_a$ is the amount of drug associated with the polymer, namely with the ionized COOH groups, $C_f$ is the concentration of the drug unassociated in the beads (being equivalent to the bulk concentration) and $V_g$ is the volume of water in the beads. Since the values of $D_i$, $C_f$ and $V_g$ are experimentally obtained, $D_a$ can be calculated from Eq. 1.

Figure 4 shows the relationship between the $D_a$ and the $C_f$, where at pH 1.0—2.0, no drug association with the

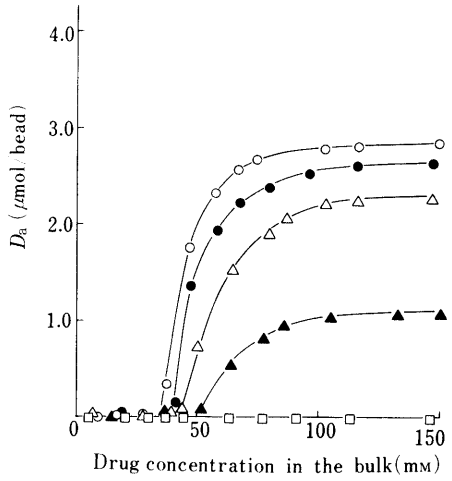


Fig. 4. Amount of Propranolol Associated with Alginic Acid Polymer Calculated by Eq. 1

Data at pH 1.0 are not shown since they are very similar to those at pH 2.0.

polymer occurred. Even at higher pHs where the COOH group should be predominantly ionized, the association seemed to be comparable to that in the acidic region up to about $C_f$ of 40—50 mM, followed by an abrupt increase of drug loading in the beads and a subsequent tendency to saturate with further increase of the drug concentration.

It should be noted here that such changes are coincident with the sudden loss of water, *i.e.* the sudden contraction of the beads and with the abrupt appearance of whitish beads. A possible explanation for what happens in the beads is as follows: the drug interacts with the anionic COOH groups, forming a complex due to electrostatic forces, for example up to about 40 mM at pH 4.0. But, when the drug concentration exceeds a critical concentration in relation to the effective concentration of the ionized COOH groups, it is considered that a water-insoluble complex precipitates in the beads. This hypothesis can reasonably explain the fact that the abrupt transformation to the whitish beads accompanied with the contraction of the beads occurred in a very narrow range of the drug concentration. It seems likely that the precipitation of the complex expels much of the water from the beads. A polarizing microscope study of the resulting whitish beads, however, did not give images typical of crystalline character. The water-insoluble complex therefore seemed to precipitate in an amorphous state rather than in a crystalline form because the molecular motion of the complex is likely to be highly restricted in the gel structure.

As can be seen in Fig. 4, the maximal amount of the associated drug was dependent on the pH as follows: 1.1 μmol/bead for pH 2.5, 2.2 for pH 3.0, 2.7 for pH 3.5 and 2.8 for pH 4.0. Additionally, all of the beads contain a basic amount of the unassociated drug in the minimal water content (see Fig. 2).

In the case where the drug is maximally loaded, a complex equilibrium must exist among the dissociation of the COOH groups, the association of the drug with the polymer, the precipitation of the complex and the hydration of the gel structure. Although there is no knowledge of the association constant of the drug with the polymer, the results suggest that the anionic COOH groups play an

important role in determining the loading capacity of a cationic drug such as propranolol, and the total amount of the drugs in the beads may be controlled by adjusting the pH of the medium. Such amorphous precipitation is likely to offer an efficient reservoir for the release of the loaded drugs.

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