Preparation and Drug Retention of Biodegradable Chitosan Gel Beads

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Chitosan (CS) gel beads containing drug could be prepared in amino acid solutions of pH about 9, despite the requirement for a pH above 12 for gelation in water. This phenomenon was observed not only in amino acid solutions but also in solutions of compounds having amino groups. A solute concentration of more than 10% was required for preparation of gel beads at pH 9. Gelation of the CS beads required about 25 to 40 min, depending on the species of amino acid. Lidocaine hydrochloride (LC) as a model drug was retained in the beads to about 20 to 35% of the theoretical total amount, despite being a water-soluble drug. The release of LC from the CS gel beads was prolonged. The release pattern was not affected by the species of amino acid or CS, or the preparation time.

Key words chitosan; gel; biodegradability; amino acid

The polysaccharide chitosan (CS) is known as an excellent material for drug preparation. CS is a plentiful natural biopolymer and is also non-toxic, biocompatible and biodegradable.[1—4] These properties are important for materials that are implanted in the body, because such materials must avoid the host's defense system during their long-term contact with living structures.[5] CS has been studied as a unique vehicle for the sustained delivery of drugs; in addition, the preparation of CS microspheres has been examined.[6—8] We are investigating the preparation of a suitable vehicle, for example micro-gel beads, for intra-articular injection in chronic rheumatoid arthritis to allow sustained intra-articular drug delivery. In this study, CS gel beads were prepared by a new method, and their drug release behavior was evaluated.

Experimental

Materials CS of various degrees of deacetylation [70% (7B), 80% (8B), 90% (9B), and 100% (10B)] were purchased from Katokichi Co. Ltd. (Japan) (Table 1). Lidocaine hydrochloride (LC) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Egg-white lysozyme (activity 0.8 mg/mg) was purchased from Wako Pure Chemical Industries (Japan). The other reagents were obtained from Wako Pure Chemical Industries (Japan) and Nacalai Tesque Inc. (Japan).

Enzymatic Degradation of CS 0.5% CS solution (180 ml) in 0.1 M acetate buffer (pH 4.5) containing 0.2 M sodium chloride was prepared and incubated at 37 °C. Preincubated lysozyme solution (about 4 mg/ml) in physiological saline was added to make up to 20 μg/ml lysozyme. The viscosity of the solution was measured periodically at 37 °C by using a B type viscometer (Tokyokeiki). The enzymatic degradation of CS polymers was also confirmed by determination of low molecular weight CS in the presence of lysozyme (5 mg/ml) by an improvement of the method of 'Elson–Morgan reaction'.[9]

Preparation of CS Gel Beads CS gel beads were prepared by the following method. 1% (w/w) CS was dissolved in 0.1 M acetate buffer (pH 4.5) or in 1% of various weak acids. Then 1% LC, a water-soluble model drug, was added to the CS solution. Three grams of the solution, theoretically containing 30 mg of drug, was dropped slowly into 30 ml of 10% amino acid solution using a pipette. Hydrogel beads were formed spontaneously. The dried gel beads were obtained by drying the hydrogel beads at 37 °C and at room temperature *in vacuo*.

Gelation Time The gelation of CS gel beads was investigated according to the method of Shiraishi *et al.*[10] Cresol red, which shows a yellow color below pH 8.8 and a red color above pH 8.8, was added to the CS solution in 0.1 M acetate buffer (pH 4.5) to monitor its gelation. The gel beads containing cresol red initially showed a yellow color because the pH of the CS solution was 4.5. The color changed from yellow to red as the amino acid solution (pH 9.0) diffused into the beads.

Dissolution Test Release of LC from the various types of CS gel beads into 0.1 M phosphate buffer (pH 7.2) was determined. The dried gel beads were added to 50 ml of the dissolution medium in a 100 ml sample bottle and shaken in a bath incubator shaker at 37 °C. A 4 ml aliquot of the solution was removed periodically for analysis and replaced with 4 ml of the dissolution medium (pre-warmed to 37 °C) to maintain a constant volume. The absorbance of each sample was determined on a Hitachi model 200-20 spectrophotometer at 262 nm. All the dissolution tests were performed in triplicate.

Results and Discussion

Enzymatic Degradation of CS In general, mucopolysaccharides are degraded by enzymatic hydrolysis; the degradation of CS has been reported.[1—5] Initially, the degradability of CS having various degrees of deacetylation was investigated. The extent of degradation was reflected by a lowering of viscosity. As shown in Fig. 1, certain CS polymers were degraded gradually. The extent of degradation was inversely proportional to the degree of deacetylation, and the 100% deacetylated CS (10B) was not degraded. There was no change in viscosity when lysozyme was not added. Similar results were also obtained when degradation was assessed by determination of low molecular weight CS degradation products liberated from the original CS polymer by lysozyme.

Preparation of CS Gel Beads CS forms a gel in solutions with pH above 12. Previously, CS gel beads have been prepared in such a solution. However, when a drug was present in the CS solution, the gel beads could not maintain a spherical shape. Preparations made at a lower pH are preferable in terms of their effects on the solubility or the stability of the drug contained in the gel beads and on the tissue into which they are injected. In solutions containing amino acids [glycine (Gly), alanine (Ala), sodium aspartate (Asp-Na), or

Table 1. Characteristics of CS

CS species	Degree of deacetylation (%)	Molecular weight[a)]	Viscosity[b)] (cps)
7B	70	2210000	66
8B	80	2140000	52
9B	90	900000	24
10B	100	950000	9

a) Reference 2. *b*) Determined on 0.5% CS solution at 37 °C by using a B type viscometer (Tokyokeiki).

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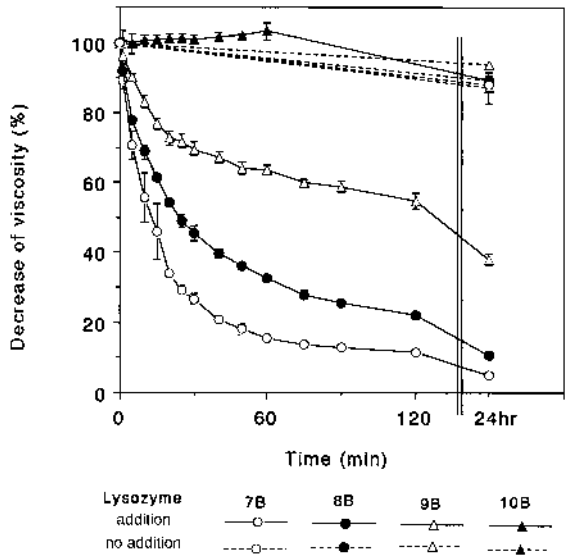

Fig. 1. Change of Viscosity of CS Solution in the Presence of Lysozyme

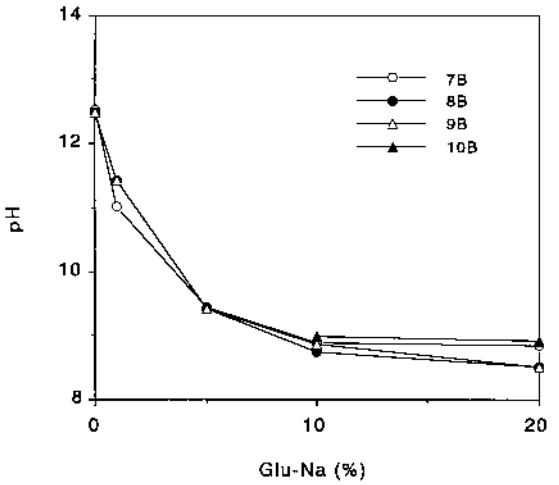

Fig. 2. Effect of Glu-Na Concentration on the pH of Gelation

sodium glutamate (Glu-Na)], CS gelated at about pH 9. This phenomenon was not affected by the species of salt (succinic acid, citric acid, lactic acid, malic acid, asparagic acid, glutamic acid, or acetic acid) used to dissolve the CS. Furthermore, the gelation of CS at about pH 9 was observed not only in amino acid solutions but also in solutions of compounds having amino groups (ammonium chloride, dimethylammonium chloride, trimethylammonium chloride, *etc.*). Figure 2 shows the effect of Glu-Na concentration on the pH of gelation of CS. The pH at which gelation occurred was decreased as the concentration of amino acid (or compound with an amino group) increased. A concentration of more than 10% was required to prepare gel beads at the lowest pH. CS solutions containing more than 1% of LC could not maintain the spherical shape of the gel beads. On the other hand, the 100% deacetylated CS (10B) could not gelate at Glu-Na concentrations less than 10% despite increasing pH. Similar results were obtained when other amino acids were added to the solution instead of Glu-Na.

Gelation Time The time was usually determined by complete change of the internal color of the gel beads containing cresol red from yellow to red. At pH 9.0 the time was affected by the species of amino acid in solution, as shown in Fig. 3. The gelation of the CS gel beads required 25 min in 10% Gly solution, and 40 min in 10% solutions of the other amino acids (Ala, Asp-Na, Glu-Na). Before this time, the gel beads could not be removed from the amino acid solution because they could not maintain a spherical shape in the process of removal or drying. Gel beads prepared with 9B or 10B could not maintain a spherical shape when removed from the solution, despite the color of the inner gel matrix changing to red.

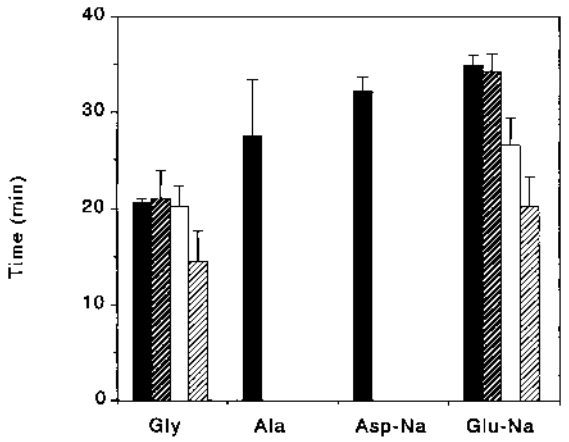

Fig. 3. Effect of Amino Acid Species on the Time of Gelation
7B, ■; 8B, ▨; 9B, □; 10B, ▨.

Retention of LC LC was selected as a model drug. It is one of the most difficult drug on the retention in the gel beads, because LC is adequately soluble, and dose not form complex with CS in the condition of the preparation of the gel beads. We thought, if LC could retain in the CS gel beads, other drugs would be able to retain easy in the gel beads. Three grams of CS solution (pH 4.5 in 0.1 M acetate buffer) containing 1% LC was dropped slowly into 30 ml of 10% amino acid solution (pH 9.0) using a pipette and left at room temperature for 40 min (or 25 min for glycine). The leakage of LC from gel beads was not affected by the species of amino acid, but was affected by the soaking time in the amino acid solution. Leakage increased as the soaking time increased, because LC is very soluble in water. The amount of LC retained in the gel beads was calculated as the theoretical total amount minus the amount of leakage. LC was retained in the gel beads to an extent of about 20 to 35% of the theoretical total amount. This result is due to the solubility of LC in the solution. The retention of drug in the gel beads may be increased if insoluble drug is used.

Release Profile of LC from CS Gel Beads Sustained release of LC from CS gel beads prepared with amino acids was observed. The LC retained within the beads was released gradually, and essentially all of the LC was released after 60 min (Fig. 4). The release pattern of LC from gel beads was not affected by the species of amino acid. However, with the gel beads prepared using Ala, Ala crystallized at the surface of the gel beads after drying. The standard deviation of LC release from the gel beads prepared using Ala was large. The LC release pattern was virtually unaffected by increasing the preparation time or changing the species of CS (7B, 8B). The solubility of LC decreases in pH 9 amino acid solution

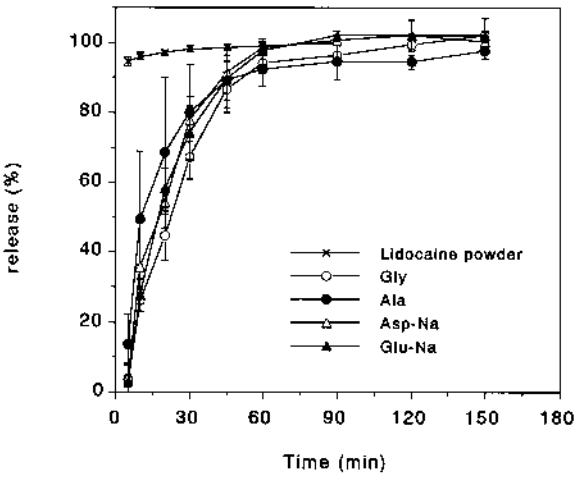

Fig. 4. Effect of Amino Acid Species on LC Release from CS (7B) Gel Beads

Soaking time: 40 min.

because of the formation of free lidocaine from LC. This decrease in drug solubility may result in a delayed release of LC from gel beads. However, both LC and free lidocaine are adequately soluble in the test solution. Thus, the sustained release cannot be attributed to the insolubility of the drug, but rather to its retention in the CS gel matrix.

CS gel beads have been suggested as a possible vehicle for sustained drug delivery. Changing the gel matrix by utilizing complex formation between CS and other mucopolysaccharides, such as chondroitin sulfate or hyaluronic acid, may be more effective for controlling the release of drug. However, the degradability of CS will also be affected by other enzymes, body constituents and application conditions. Further studies of the effects of these factors should be performed.

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