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Research paper

Use of floating alginate gel beads for stomach-specific drug delivery

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Abstract

Two types of alginate gel beads capable of floating in the gastric cavity were prepared. The first, alginate gel bead containing vegetable oil (ALGO), is a hydrogel bead and its buoyancy is attributable to vegetable oil held in the alginate gel matrix. The model drug, metronidazole (MZ), contained in ALGO was released gradually into artificial gastric juice, the release rate being inversely related to the percentage of oil. The second, alginate gel bead containing chitosan (ALCS), is a dried gel bead with dispersed chitosan in the matrix. The drug-release profile was not affected by the kind of chitosan contained in ALCS. When ALCS containing MZ was administered orally to guinea pigs, it floated on the gastric juice and released the drug into the stomach. Furthermore, the concentration of MZ at the gastric mucosa after administration of ALCS was higher than that in the solution, though the MZ serum concentration was the same regardless of which type of gel was administered. These release properties of alginate gels are applicable not only for sustained release of drugs but also for targeting the gastric mucosa.

Keywords: Alginate gel bead; Chitosan; Floating; Intragastric drug delivery; Metronidazole

1. Introduction

Calcium-induced alginate gel beads (Alg–Ca) have been developed in recent years as a unique vehicle for drug delivery. Alg–Ca is rapidly formed by gelation of alginic acid in the presence of calcium ions and is able to incorporate some compounds such as drugs or polysaccharides in the gel matrix [1,2]. The beads have been used in various ways in the gastrointestinal tract, for example, for sustained release of drugs or to adsorb bile acid [3]. Other studies have been made of gastroretention in attempts to improve control over drug release or to achieve a site-specific delivery [4–6]. The role of mucoadhesives and floating properties of the alginate gel forms as well as the effect of varying dosage have been investigated. The floating system has been found to be particularly promising for drug gastroretention [7,8]. For example, lyophilized calcium alginate prolonged gastroretention in human volunteers [9].

In this study, we prepared two types of Alg–Ca that, when orally administered, were capable of floating in gastric juice. One is a hydrogel bead whose buoyancy is attributable to vegetable oil held in the alginate gel matrix. The other is dried Alg–Ca containing chitosan (CS) which contains sufficient air to provide buoyancy. Drug release profiles of both were tested in acidic solution (using drugs that do not decompose under acidic conditions). The drug release profiles from buoyant Alg–Ca were also determined in artificial gastric juice. Gastroretention with drug release may be an advantageous strategy for *Helicobacter pylori* eradication in the stomach mucosa. With this aim in mind, metronidazole (MZ) was selected as a model drug for incorporation in Alg–Ca. It has been widely used to prevent recurrence of peptic ulcer disease, a phenomenon which is correlated with infection by *H. pylori* [10]. We investigated not only the release of MZ but also its delivery to the stomach mucosa.

2. Materials and methods

2.1. Materials

MZ was obtained from Sigma (MO) and sodium alginate (Alg–Na) purchased from Nacalai Tesque (Kyoto). One CS, (fine powder, molecular weight; MW 30 000, degree of deacetylation; DA 85%), CS(F) was obtained from Kimitsu Chemical Industries (Tokyo) and another (CS-500) from Wako Pure Chemical Ind. (Osaka). Viscosity of each 1% CS-1% lactic acid solution was measured by

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using a Brookfield viscometer at 20°C and was found to be 9.4 cps (CS(F)), 3140 cps (CS-500), respectively. Two others, CS-7B (MW 2 210 000, DA 70%) and CS-10B (MW 950 000, DA 100%) were obtained from Funakoshi (Tokyo). CS (F) was sieved, and divided into three samples according to particle diameter (fine; <75 μm, medium; 75–180 μm, rough; 180–500 μm) to evaluate the role of particle size. Chitin was obtained from Kimitsu, curdlan and sodium dextran sulfate from Wako, and pullulan and xylan from Seikagaku Co. (Tokyo). Olive oil and corn oil were obtained from Wako Pure Chemical Ind., and sesame oil from Nacalai Tesque. All other chemicals used were standard high-purity materials obtained from commercial sources.

2.2. Preparation of alginate gel bead containing vegetable oil (ALGO)

Alg–Na (1% W/W) was dissolved in distilled, demineralized water with agitation. MZ and a vegetable oil were added to the solution. This solution (2.5 g) containing MZ (250 mg) and oil (5–40% (w/w)) was dropped into 0.1 M calcium pantothenate (10 ml) and left at room temperature for a day. The resultant hydrogel beads were washed twice with 50 ml distilled water and used for each test.

2.3. Preparation of alginate gel bead containing chitosan (ALCS)

MZ (1.0 g) and 0–0.5 g of CS were dispersed in 10 g of Alg–Na (1% w/w) solution. This solution (2.5 g) was dropped into 0.1 M calcium pantothenate (10 ml) and left to stand at room temperature for a day. These hydrogel beads were washed twice with distilled water and dried at 35°C for 8 h on a dish, before being held under vacuum in a desiccator in the presence of P_2O_5.

2.4. Buoyancy of the preparations

Specific gravity of the test solution (distilled water, 0.9% NaCl or JPXIII 1st fluid) was previously measured using a standard pycnometer or electronic densimeter (Mirage Trading Co., Osaka). Sample beads (ten granules) were steeped in 50 ml of each test solution and their buoyancy was observed visually. The preparation was considered to have buoyancy in the test solution only when all of the granules floated in it.

2.5. Study of morphology and particle size of ALCS

The particle sizes ($n = 20$) of Alg–Ca were measured with a caliper and the contraction ratio (CR) of the bead was calculated by dividing the mean volume of dried gel by that of hydrogel. The morphology of ALCS was examined by scanning electron microscopy (SEM) at the Meiji Institute of Health Science (Odawara, Japan). After the sample had been coated with gold–palladium, it was observed with a scanning electron microscope (Hitachi S-2400, Tokyo).

2.6. Test of MZ dissolution from the preparations

The release of MZ from ALGO or ALCS in 500 ml of HCl-solution (JPXIII 1st fluid, pH 1.2) was determined using a JP XIII dissolution test apparatus (Toyama Sangyo Co., Tokyo, paddle method, 37 ± 0.5°C). The rotation speed of the paddle was adjusted to 150 rev./min for homogenous dispersion of gel beads in dissolution medium. A 0.5-ml aliquot of test solution was removed periodically and 0.5 ml of new medium (37°C) added to maintain a constant volume. Each sample removed was diluted with HCl-solution and the absorbance measured at 277 nm using a spectrometer UV-1200 (Shimadzu, Kyoto). All dissolution tests were performed in triplicate.

2.7. Animal study

This experimental protocol was approved by the Ethics Committee at Hokuriku University. ALCS containing MZ was administered orally (15 mg/kg) to guinea pigs (Hartley, male, 500–600 g) fasted for 2 days. After 2–4 h, the animals were anesthetized by intraperitoneal injection of nembutal. The abdominal skin was opened and blood was collected from the vena cava caudalis leading to death by exsanguination. Blood was centrifuged in a refrigerated ultracentrifuge (Hitachi CF7D, 4°C) at 3600 rev./min for 10 min. The serum was separated and then deproteinized by adding methanol, followed by centrifugation. The supernatant was removed and the concentration of MZ determined by HPLC, as follows [11]. The system comprised an LC-6A pump (Shimadzu), a packed column (Cosmosil, Nacalai Tesque, Kyoto, 150 × 4.6 mm (i.d.)), and a SPD-6A variable UV detector (Shimadzu). Chromatography was conducted at ambient temperature using an eluent comprising 85%(V/V) 10 mM phosphate buffer (pH 5.5) and 15%(V/V) methanol at a flow rate of 0.8 ml/min with the detector wavelength set at 320 nm. At the time of sacrifice, the guinea pig stomach was removed, opened longitudinally, and the remaining ALCSs were taken out and placed on a filter paper. The beads were then soaked in HCl solution and the MZ eluted from them was measured by HPLC. Alternatively, the stomach was gently rinsed in 30 ml of Sörensen -buffer (pH 7.4) three times and spread on a glass plate. The mucosal surface was scraped gently with a glass slide and the top layer separated from the muscular layers [12]. The removed mucosa was mixed with 3 ml of Sörensen-buffer in a glass tissue grinder. After being ground, the homogenate was centrifuged in a refrigerated ultracentrifuge at 3000 rev./min for 10 min. The supernatant was removed and filtered through a 0.2 μm filter. The amount of MZ contained in the sample was then measured by HPLC. (If necessary, data were compared using Student's two tailed *t*-test and the difference was considered significant when $P < 0.05$).

3. Results and discussion

3.1. Buoyancy of ALGO

When MZ-free Alg–Ca was steeped in water, physiological saline or HCl solution that mimics gastric juice, it sank as shown in Table 1. However, the ALGO containing about 8% MZ and 30% olive oil floated in these solutions. The same degree of buoyancy resulted if a 30% concentration of other vegetable oils was used. When ALGO containing 10 or 20% oil was steeped in water, it first sank, then gradually released MZ and floated. ALGO containing 40% olive oil was not used because at that concentration oil began to leak from the beads.

Table 2 shows the amounts of MZ loaded in Alg–Ca or ALGOs. All ALGOs demonstrated a higher loading capacity for MZ than Alg–Ca.

3.2. Buoyancy of ALCS

ALCS (5% CS) floated in physiological saline or HCl solution. If the percentage of CS was 3% or lower, the beads no longer floated in any of the solutions. And floating was not observed for Alg–Ca containing 5% chitin, curdlan, sodium dextran sulfate, pullulan or xylan, instead of chitosan. The percentage of CS barely affected the amount of the drug that could be incorporated in ALCS. A 160–170 mg loading of MZ ALCS was achieved, about 65% of the amount of MZ added. The buoyancy was not affected by the molecular weight or deacetylation ratio of the CS added. Also, the buoyancy was not affected by variation in the particle diameter of CS contained in ALCS.

3.3. Morphology of ALCS

The diameter of dried Alg–Ca containing each polysaccharide and the CR are shown in Table 3. ALCS was larger than Alg–Ca, which contains polysaccharides other than CS. The CR of ALCS was about 0.4, though CRs of the others were below 0.3. Fig. 1 shows scanning electron micrographs (SEMs) of dried Alg–Ca and ALCS. The surface of ALCS is uneven compared with that of Alg–Ca. SEMs of the surface of sectioned ALCS show that many large pores are present in the gel matrix. These results show that the contraction of alginate gel matrix caused by water evaporation is depressed by the presence of CS.

Table 1
Buoyancy of ALGO[a]

	Water	0.9% NaCl	1st fluid
(Specific gravity)	(1.007)	(1.014)	(1.013)
Olive oil (%)			
0 (Alg–Ca)	S	S	S
5	S	S	S
10	F	F	F
20	F	F	F
30	F	F	F
30[b]	F	F	F

[a] S, sink; F, float.
[b] Containing 190 mg metronidazole.

Table 2
Amount of MZ loaded in ALGO (2.5 g hydrogel)[a]

Olive oil (%)	MZ (mg)
0 (Alg–Ca)	164.0 ± 5.0
5	180.0 ± 5.0
10	187.0 ± 7.2
20	192.0 ± 5.9
30	190.0 ± 4.2
40	183.0 ± 1.5

[a] The data are presented as mean value ± SD ($n = 3$).

3.4. Profiles of MZ release from ALGO

MZ was released rapidly from Alg–Ca in HCl solution, with no more being released after 30 min (Fig. 2). Conversely, MZ contained in ALGO was released only gradually into the solution. The release rate decreased with the increase in the amount of olive oil. For example, ALGO containing 30% olive oil remained floating throughout the experiment, and only about 80% of the drug had been released after 60 min. Also ALGO did not always disintegrate.

3.5. Release profiles of MZ from ALCS

ALCS released MZ slowly in HCl solution as shown in Fig. 3. Twenty percent of MZ had been released 10 min after exposure of the ALCS to the solution, and all had been released by about 90 min. These were virtually identical in spite of the different percentages of CS. This was supported by using the Higuchi-plot method to estimate the release rate constants for ALCS and Alg–Ca [13] (graphs not shown). The values calculated from the data ($<80\%$) were 14–15%/min$^{1/2}$. These results show that the many pores within ALCS accelerated the permeation of the test solution into ALCS. MZ then diffused into the gel matrix even though an increase in diameter extended the diffusion distance before MZ was released from the vehicle.

Table 3
Diameters of dried alginate gel beads containing 5% polysaccharide

Polysaccharide	Diameter[a] (mm)	CR
None (Alg–Ca)	2.4 ± 0.1	0.28
Agar	2.8 ± 0.2	0.26
Pullulan	2.5 ± 0.2	0.19
Xylan	2.7 ± 0.2	0.22
Chitin	2.7 ± 0.2	0.27
Chitosan (ALCS)	3.4 ± 0.1	0.39

[a] The data are presented as mean value ± SD ($n = 20$).

At the end of an experimental period, it was observed that ALCS had swelled and remained buoyant. Similar drug release profiles were obtained for different types of ALCS. Further, release profiles were similar when the particle diameter of CS contained in ALCS changed (data not shown).

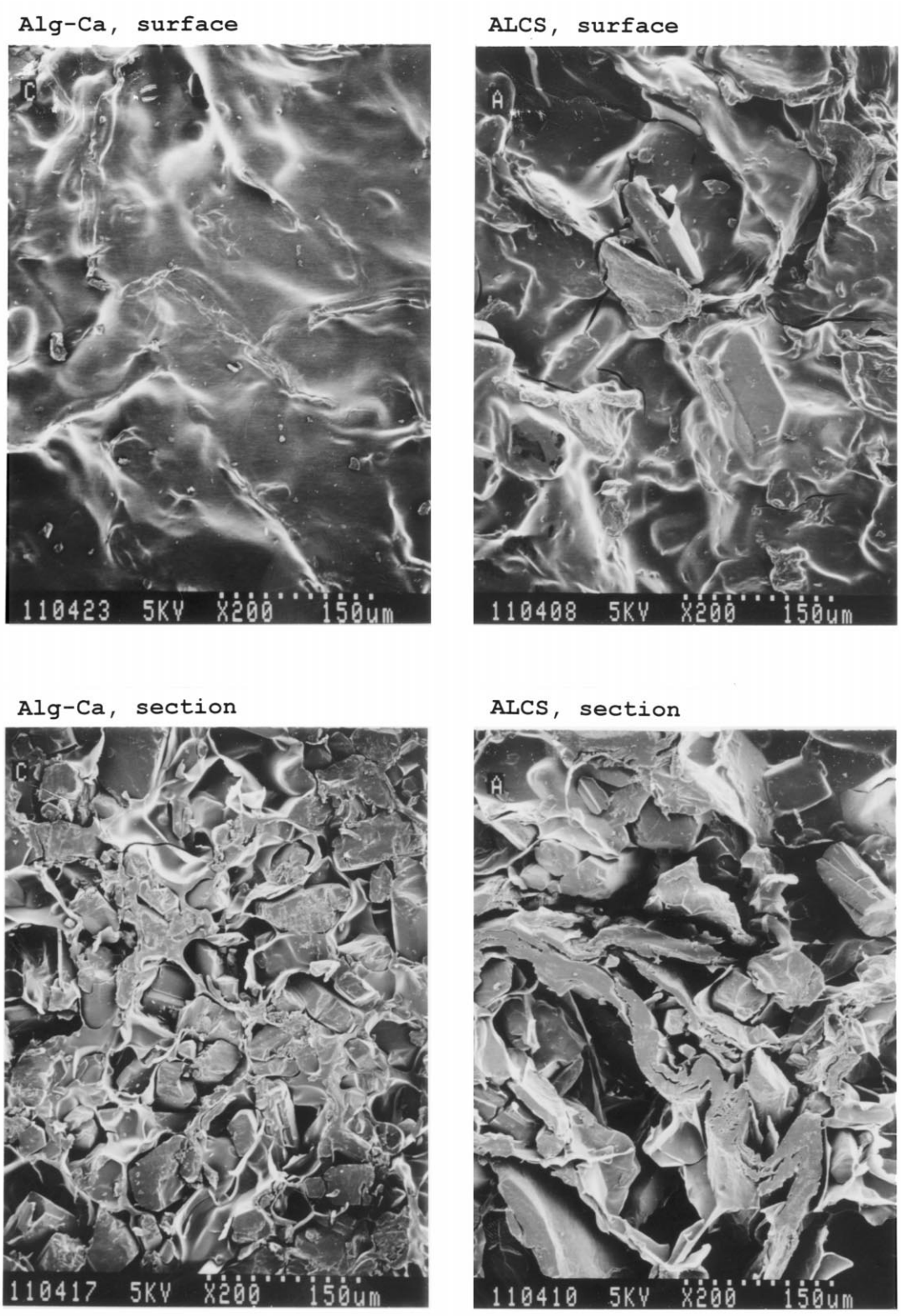

Fig. 1. SEM micrographs of Alg–Ca and ALCS (5% CS).

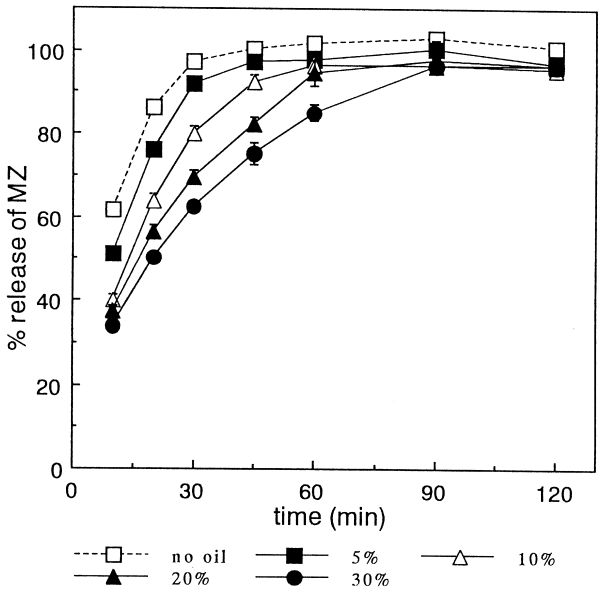

Fig. 2. Profiles of MZ release from ALGO (0–30% olive oil).

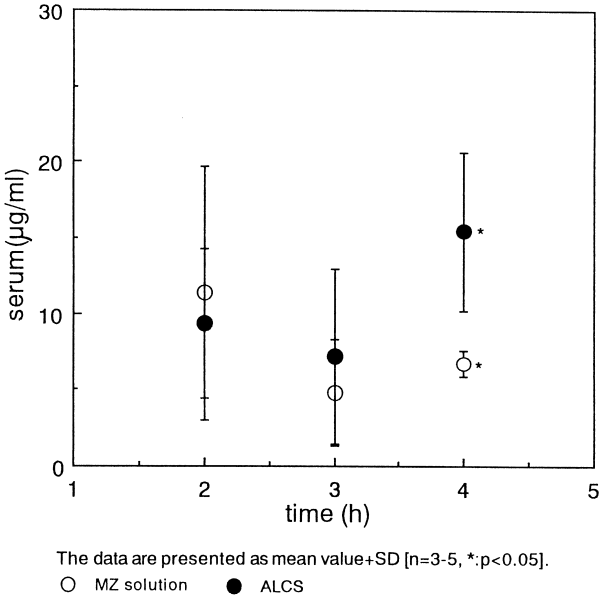

Fig. 4. Serum concentrations of MZ after oral administration to guinea pigs.

3.6. Animal study

When MZ solution was administered orally to guinea pigs (20 mg/kg), the serum concentration was 11.4 ± 8.3 μg/ml after 2 h, as shown in Fig. 4. After 3–4 h they were about 5 μg/ml. MZ is immediately absorbed from the gastrointestinal cavity, but only a small amount reaches the gastric mucosa. When MZ solution is administered, MZ is absorbed mainly from the small intestine and reaches the gastric mucosa via the circulatory system. Therefore, the delivery of MZ to the gastric mucosa is dependent on the blood concentration. After administration of MZ solution, all of the mean MZ amounts after 2, 3 or 4 h were about 0.1–0.2 μg (/cm^2 stomach) regardless of the difference in the blood concentration, as shown (Figs. 4 and 5). After ALCSs containing MZ are administered orally, they release the drug in the stomach by floating on the gastric juice. Their capacity to float in the stomach of the guinea pig was confirmed visually upon excision. The amount of MZ reaching the gastric mucosa after administration (2 or 3 h) of ALCS was greater than that in the solution, although the serum concentration of MZ did not change. The concentration was maintained for 4 h as shown in Fig. 4, and this may be attributable to the sustained release of MZ from ALCS. The residual amount

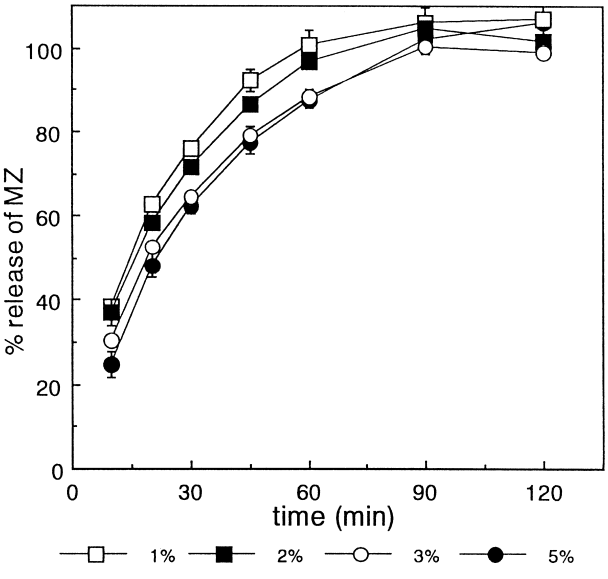

Fig. 3. Profiles of MZ release from ALCS (1–5% CS).

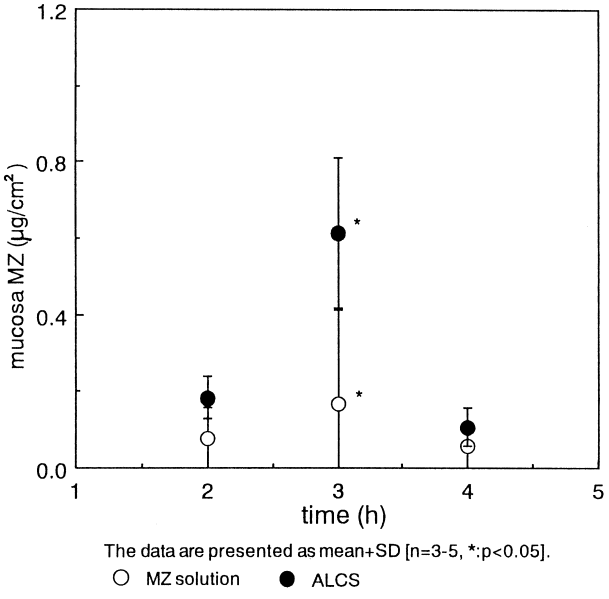

Fig. 5. MZ amount in the gastric mucosa after oral administration to guinea pigs.

of MZ in ALCSs taken from the stomach after 3 h was less than 5%. In addition, we did not try to administer ALGO to guinea pigs because the hydrogel beads were too large (about 4 mm diameter) for them to swallow.

4. Conclusion

In this study, we prepared two types of buoyant alginate gel beads and examined their drug release profiles. Both types, ALGO and ALCS, floated in the acidic environment of the gastric fluid if they contained a vegetable oil or air in their gel matrix and gradually released the model drug, MZ. These properties are applicable not only to the sustained release of drugs but also to the targeting of the gastric mucosa. The floating alginate gel appears to be a promising vehicle for delivering such preparations specifically to the region [14,15]. And it will play an important role in therapy of diseases in which a gastric-mucosa-specific drug delivery regimen should be considered, such as *H. pylori* infection.

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