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# In vitro and in vivo evaluation of mucoadhesive microspheres consisting of dextran derivatives and cellulose acetate butyrate

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**Abstract**

The objective of this study was to evaluate mucoadhesive properties and gastrointestinal transit of microspheres made of oppositely charged dextran derivatives and cellulose acetate butyrate (CAB). The microspheres were prepared by emulsion solvent evaporation method. A reference microsphere was made of lactose instead of dextran derivatives. Microspheres with a diameter of 425–710 μm were examined for in vitro mucoadhesion by the everted sac method. The results indicated that the percentage of adherence to the rat small intestine was affected by the amount of dextran derivatives in the microspheres. After 1.5 h, the adhering percent of the reference microspheres and the microspheres containing 50% of dextran derivatives were 34 and 74%, respectively. Then gastrointestinal transit after oral administration to rats was evaluated by counting the microspheres remaining in the stomach and small intestine. The microspheres containing 40% of dextran derivatives adhered to the stomach rather than the small intestine. Mathematical analysis revealed that the time required for 50% of microspheres to leave the stomach was 1.42 h, three times longer than the reference. These findings suggest that the microsphere is a promising device as a multiple-unit mucoadhesive system.


*Keywords:* Dextran derivatives; Mucoadhesion; Microspheres; Gastrointestinal transit; Polyion complex; Cellulose acetate butyrate

## 1. Introduction

The use of natural polymers in formulation design of dosage forms has received considerable attention, especially from the viewpoint of safety. Among these polymers, dextrans are naturally occurring non-toxic water-soluble polysaccharides, which have been used in the medical field. Hydrophilic dextran derivatives are also used clinically. For instance, [2-(diethylamino) ethyl] dextran (EA) is an anion-exchange resin that is used as a hypolipidaemic agent and dextran sulfate (DS) is used as an anticoagulant and as a hypolipidaemic agent (Reynolds, 1993).

However, few studies have reported the potential of dextrans for delivery of drugs orally. The main limitation of dextrans for the preparation of oral sustained release systems arises from its rapid dissolution in aqueous environments leading to fast drug release. In order to overcome this problem, we used physical

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cross-linking of hydrophilic polymers for long-term drug release. Hydrophilic dextran derivatives have already been proposed as useful excipients for a sustained release matrix tablet (Miyazaki et al., 1993). The mixtures of oppositely charged dextran derivatives react with each other to form polyion complexes when swollen. The formation of the complex reduced the drug release rate from hydrophilic matrix tablets. From a clinical viewpoint, particulate systems offer obvious advantages over single-unit dosage forms. For instance, they spread over a large area of the gastrointestinal tract, and have a lower risk of dose dumping. A particulate hydrophilic matrix made of dextran derivatives, however, could not depress drug release because of its rapid swelling and low mechanical strength. Thus, we developed controlled release microspheres prepared from a mixture of oppositely charged dextran derivatives strengthened by cellulose acetate butyrate (CAB; Miyazaki et al., 2003, in press). The drug was released gradually through the polyion complex matrix incorporated into a hydrophobic polymer matrix. The most delayed drug release was obtained when DS/EA mixture was used as oppositely charged dextran derivatives.

On the other hand, dextran derivatives have good mucoadhesive properties, and no reduction of mucoadhesion is caused by polyion complex formation (Miyazaki et al., 2002). Therefore, the mixtures of oppositely charged dextran derivatives are expected to be useful as mucoadhesive agents for drug delivery systems. Recently, mucoadhesive dosage forms have received much attention because their properties lead to increased residence time of the device in the gastrointestinal tract (Helliwell, 1993), which will most likely relate to increased bioavailability of the encapsulated drug.

In the present study, we evaluated microspheres consisting of DS/EA mixture and CAB for their mucoadhesive properties, and investigated their gastrointestinal transit after oral administration to rats.

## 2. Materials and methods

### 2.1. Materials

Dextran sulfate (DS; MW 500,000), [2-(diethylamino)ethyl] dextran (EA; MW 500,000) and theophylline (TH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Lactose (Lac) was purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). These chemicals were used after sieving through a 200-mesh sieve (<75 μm). Cellulose acetate butyrate (CAB; MW 30,000) was obtained from Fluka Chemical Co. (Buchs, Switzerland). Sugar ester DK F-10 used as a surfactant was kindly supplied by Daiichi Kougyou Seiyaku Co., Ltd. (Kyoto, Japan). All other chemicals and solvents were reagent grade and used as received.

### 2.2. Preparation and characterization of microspheres

Microspheres were produced by emulsion solvent evaporation method, and described as follows. TH, DS, and EA were dispersed in 15 ml of acetone containing 1 g of CAB, and then poured into 150 ml of liquid paraffin containing 1% (w/v) of sugar ester, at 20 °C under agitation (300 rpm). The mixture was mechanically stirred under atmospheric pressure to form a w/o emulsion. After 30 min, the solution was heated to 50 °C to evaporate acetone. Then the solution was gradually cooled at 20 °C and then decanted off. The removal of residual oil was performed by washing the microspheres with 50 ml of *n*-hexane three times. The microspheres were dried under vacuum at room temperature.

Particle size analysis was performed on dried microspheres by sieving through a set of standard sieves from the pharmacopoeia of Japan, 14th edition (JP XIV). The microspheres sieved at 425–710 μm were used for further experiments.

Drug content of microspheres was determined as follows. Approximately 10 mg (accurately weighted) of microsphere was dissolved (or dispersed) in 50 ml of methylene chloride. The solution was subjected to sonication at room temperature for 10 min. The resultant solution was filtered through a 0.2 μm membrane filter and analyzed spectrophotometrically at 274 nm. All drug content determinations were performed in triplicate.

### 2.3. Image of swelling of microspheres

A microsphere settled on a slide glass was wetted by three drops of water. After 1 and 5 min, the

process of swelling was observed using CCD camera (VH-5900, Keyence, Tokyo).

### 2.4. In vitro mucoadhesive study

The in vitro mucoadhesive test was carried out using a small intestine isolated from rats. Fasted rats (300–350 g, male, Spraque–Dawley strain, Sankyo Labo, Co., Ltd.) were sacrificed, and then small intestinal tissue was excised and flushed with saline. Five centimeter segments of jejunum were everted using a glass rod. Ligatures were placed at both ends of the segment. One hundred microspheres were scattered uniformly on the everted sac from the position of 2 cm above. Then the sac was suspended in a 10 ml tube containing 8 ml of saline by the wire, to immerse in the saline completely. The sacs were incubated at 37 °C and agitated horizontally. The sacs were taken out of the medium after immersion for 0.5, 1, 1.5, 2, and 2.5 h, immediately repositioned as before in a similar tube containing 8 ml of flesh saline and unbound microspheres were counted. The adhering percent was presented by the following equation:

$$\text{Adhering}\,\% = \frac{100 - \sum_{t=0} N_t}{100} \times 100 \qquad (1)$$

where $N_t$ is the number of unbound microspheres at the time $t$ after incubation. In the present study saline was used without adjustment or buffering pH because mucoadhesive properties of DS/EA mixture were not affected by pH in the medium (Miyazaki et al., 2002).

### 2.5. Gastrointestinal transit of microspheres

Male Spraque–Dawley rats weighing 300–350 g (Sankyo Labo, Co., Ltd.) were fasted for 24 h before the experiments. One hundred microspheres were orally administrated to each rat using the polyethylene tube attached to a gastric sonde with 0.2 ml of water. At specified time intervals, the rats were sacrificed with ether, and the stomach and the small intestine were removed. The small intestine was further cut into three equal segments and opened longitudinally. Then the microspheres in the stomach and each intestinal segment were counted.

### 2.6. Statistical analysis

Statistical analysis was performed with the Student's unpaired $t$-test. A difference was considered to be statistically significant when the $P$ value was less than 0.05.

## 3. Results

The microspheres used in this study are listed in Table 1. All microspheres consisted of hydrophilic and hydrophobic agents. The hydrophilic polymers, after swelling, play a role of drug release and the hydrophobic polymer strengthens the hydrophilic matrix. MS was the reference microsphere, which was made of lactose instead of dextran derivatives. MS-1, MS-2, and MS-3 consisted of 50, 40, and 30% of DS/EA mixture, respectively. The mixing ratio of DS/EA in this study was set at 1:3, because maximum complexation has been reported to occur at a weight ratio of 25:75 of DS/EA mixture (Miyazaki et al., 2001).

### 3.1. Particle characteristics of microspheres

The particle data are summarized in Table 1. The microspheres were obtained in good yields. The mean diameter of the microspheres varied between 612 and 718 μm, and the drug content was slightly greater than the loading amount. The particle size and drug content were little affected by the formulations.

### 3.2. Swelling observation

Photographs of the microspheres after 1 and 5 min of contact with water are shown in Fig. 1. After 1 min, small projections were observed on the surface of the microspheres. After 5 min, the projections grew larger and expanded out of the microspheres. It seems to be greater in order of MS-1, MS-2, and MS-3. These changes indicated that the polymer particles dispersed inside the microspheres swelled with parts reaching the surface of the microspheres and the remaining parts staying inside. These seem to be remarkably similar to previous observations for carbopol-dispersed microspheres made of polyglycerol esters of fatty acids (Akiyama et al., 1995). According to Akiyama et al., the changes as such indicated that the swollen

Table 1
Formulation and characterization of microspheres

| Sample name | CAB (g) | TH (g) | Lac (g) | DS (g) | EA (g) | Yield (%) | Mean diameter (μm) | Drug content (%, w/w)[a] |
|---|---|---|---|---|---|---|---|---|
| MS | 1.0 | 0.75 | 0.75 | – | – | 82.5 | 612 | 30.8 ± 1.5 |
| MS-1 | 1.0 | 0.25 | – | 0.313 | 0.937 | 80.6 | 673 | 12.4 ± 0.3 |
| MS-2 | 1.0 | 0.5 | – | 0.25 | 0.75 | 78.9 | 650 | 23.9 ± 1.5 |
| MS-3 | 1.0 | 0.75 | – | 0.188 | 0.562 | 77.0 | 718 | 32.1 ± 2.0 |

[a] Mean ± S.D. ($n = 3$).

polymer particles were strongly associated with the microsphere, resulting in the microspheres adhering to the mucosa leaving part of the swollen polymer particles behind within the microsphere. Therefore, our observations suggested that DS/EA mixture had excellent swelling properties and good associating function with the microsphere. In contrast, MS did not show any change in appearance even when they came in contact with water (photographs of MS are not shown).

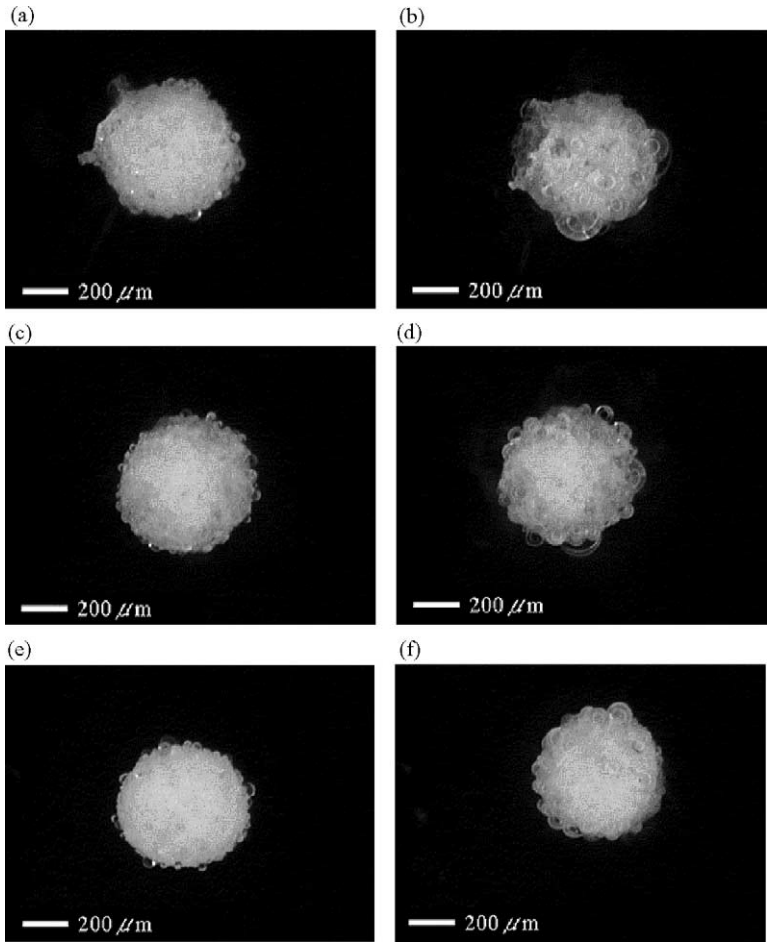


Fig. 1. Photographs of microspheres 1 min (a, c, and e) and 5 min (b, d, and f) after contact with water. (a and b) MS-1, (c and d) MS-2, and (e and f) MS-3.

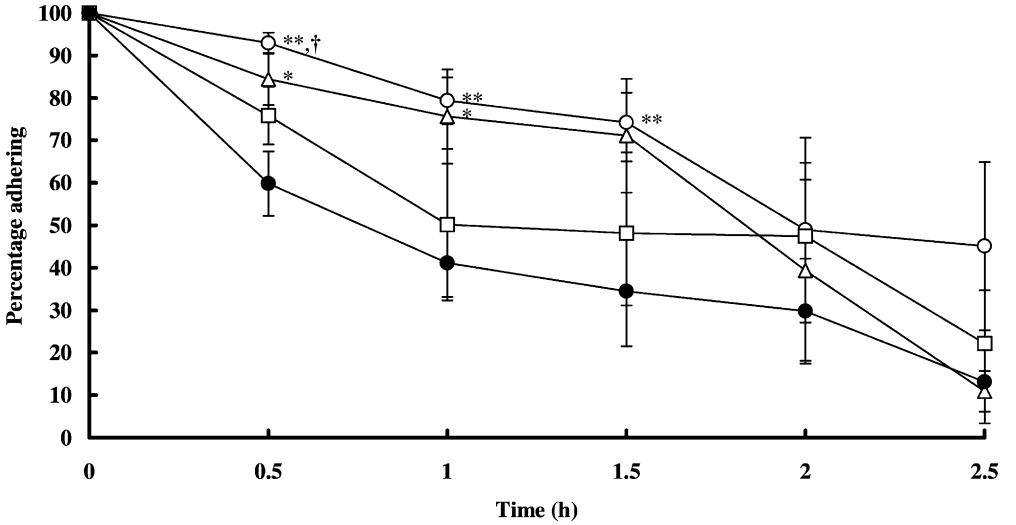


Fig. 2. In vitro mucoadhesion of microspheres to the rat small intestinal mucosa. (●) MS, (○) MS-1, (△) MS-2, and (□) MS-3. The results are represented as the mean ± S.E.M. ($n = 3$ or 4). The asterisks represents significant difference from MS, $^{*}P < 0.05$ and $^{**}P < 0.01$. †: Significant difference form MS-3 ($P < 0.05$).

### 3.3. In vitro evaluation of mucoadhesiveness

The mucoadhesive properties of microspheres were evaluated by the everted sac experiments using small intestine of rats. Since the assay is easy to reproduce and can be done in almost any laboratory (Santos et al., 1999). In addition, the time course of adhering percentage was obtained by counting at appropriate intervals. The results of the experiments were presented as percentage adhering. A high percentage of adhesion indicates that microspheres have excellent mucoadhesion to mucosal tissue. Fig. 2 shows the results of the everted sac experiments for the microspheres listed in Table 1. According to the adhering percent versus time curves, MS-3 and MS separated rapidly resulting in the adhering percent at 1 h being 50 and 41, respectively. MS-1 and MS-2 separated from the sacs gradually showing an adhering percent at 1 h of 79 and 76, respectively. Significant differences were seen between MS and MS-1 ($P < 0.01$), and between MS and MS-2 ($P < 0.05$). MS-1 showed the highest percentage of adhesion at any time during the test, followed by MS-2, MS-3, and MS until 1.5 h. These results indicated that the mucoadhesive ability was affected by the amount of dextran derivatives in the microspheres. More than 75% of MS-1 and MS-2 remained on the intestinal tube even after 1.5 h from start of the test. After that, however, MS-1 and MS-2 separated rapidly. Over-hydration and over-swelling of dextran derivatives might have resulted in decreased mucoadhesion. In the case of MS-3, swelling and expansion of the polymer were too slow with insufficient adhesion to the sac for proximate times because of low amounts of dextran derivatives in the microspheres.

### 3.4. Distribution of microspheres in gastrointestinal tract

The distribution of the microspheres in various sections of the gastrointestinal tract of rats was examined at 1, 3, and 5 h after administration as shown in Fig. 3. The stomach and small intestine were opened lengthwise and the microspheres observed directly because it was one of the most reliable methods to confirm exact microsphere quantities. After 1 h, more than 80% of MS-2 was located in the stomach, where they were distributed mainly in the glandular region of the gastric surface. After 3 h, most of MS-2 had accumulated in the lower segment of the small intestine. On the other hand, only 25% of MS were observed in the stomach and half of them were found in the middle small intestine after 1 h. After 3 h, most MS were located in the lower small intestine. Some had already passed through the small intestine. The

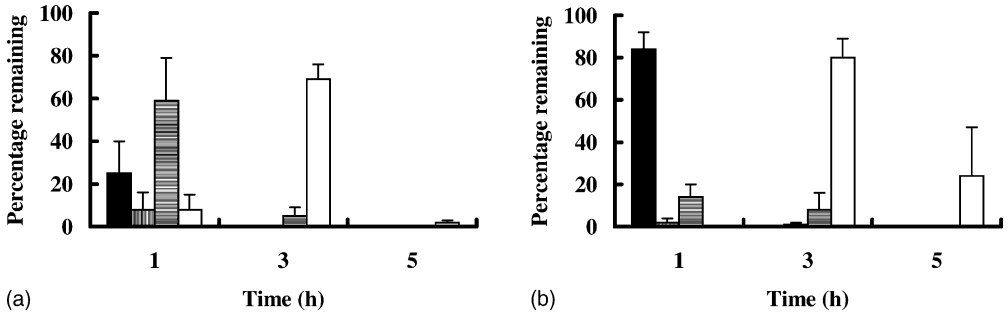


Fig. 3. Distribution of (a) MS and (b) MS-2 in the stomach and upper, middle, and lower segment of the small intestine in rats. (■) Stomach, (▥) upper segment, (▤) middle segment, and (□) lower segment of the small intestine. Data are shown as mean±S.E.M. ($n = 3$).

results indicated that the MS-2 hydrated rapidly in vivo, adhered to the mucosa of the stomach and remained there for extended periods. Thus, the gastric emptying time of MS-2 was prolonged compared with that of MS. However, 3 h later, both MS and MS-2 no longer remained in the stomach. These findings were consistent with those of in vitro mucoadhesive experiments. In vitro adhering percentage of MS and MS-2 at 1 h were 41 and 84, respectively, but those at 2.5 h had similar small values. This reduction of mucoadhesion may be caused by the dextran derivatives leaching out of the microspheres due to full hydration.

### 3.5. Evaluation of gastrointestinal transit

Transit of microspheres through the gastrointestinal tract was evaluated according to the model proposed by Akiyama et al. (1995). This model stands on the following assumptions: (i) microspheres administered to a rat were emptied from the stomach mono-exponentially after a lag time, $T_s$; (ii) transit of the microspheres through each segment of the small intestine was at zero-order; (iii) microspheres proceeded through the upper, middle, and lower segment of the small intestine in $T_u$, $T_m$, and $T_l$ hours, respectively and then reached the colon. In the model, the time at which 50% of microspheres has been emptied from the stomach is expressed as $T_{s50}$. The time at which 50% of microspheres has reached the colon is expressed as $T_{50}$. The percentage of the microspheres remaining in the stomach, $R_s$ at the time $t$ is represented as:

$$R_s = 10^2 \quad (t \le T_s) \tag{2}$$

$$R_s = 10^{2-K_s(t-T_s)} \quad (t > T_s) \tag{3}$$

where $K_s$ is defined as the first-order gastric emptying rate constant. The percentage of the microspheres remaining in the stomach and upper intestine, $R_{su}$ at the time $t$ is represented as:

$$R_{su} = 10^2 \quad (t \le T_s + T_u) \tag{4}$$

$$R_{su} = 10^{2-K_s(t-T_s-T_u)} \quad (t > T_s + T_u) \tag{5}$$

The percentage of the microspheres remaining in the stomach, upper, and middle intestine, $R_{sum}$ at the time $t$ is represented as:

$$R_{sum} = 10^2 \quad (t \le T_s + T_u + T_m) \tag{6}$$

$$R_{sum} = 10^{2-K_s(t-T_s-T_u-T_m)} \quad (t > T_s + T_u + T_m) \tag{7}$$

The percentage of the microspheres remaining in the stomach, upper, middle, and lower intestine, $R_{suml}$ at the time $t$ is represented as:

$$R_{suml} = 10^2 \quad (t \le T_s + T_u + T_m + T_l) \tag{8}$$

$$R_{suml} = 10^{2-K_s(t-T_s-T_u-T_m-T_l)} \quad (t > T_s + T_u + T_m + T_l) \tag{9}$$

Then, the time required for 50% of the microspheres to be emptied from the stomach, $T_{s50}$ and the time required for 50% of the microspheres to arrive at the colon, $T_{50}$ are represented as:

$$T_{s50} = T_s + \frac{2 - \log 50}{K_s} \tag{10}$$

and

$$T_{50} = T_s + T_u + T_m + T_l + \frac{2 - \log 50}{K_s} \tag{11}$$

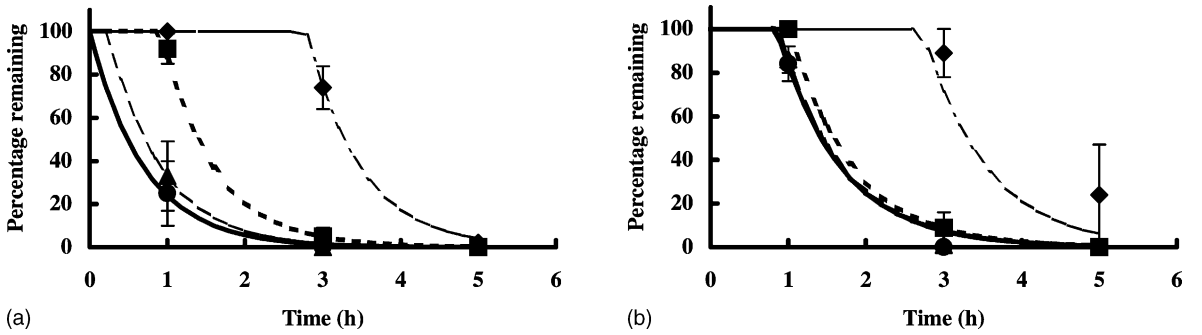


Fig. 4. Percentage of (a) MS and (b) MS-2 remaining in the stomach (●), stomach and upper small intestine (▲), stomach and upper and middle small intestine (■), and stomach and upper, middle, and lower small intestine (◆) with computer-generated gastrointestinal transit profiles. (—) stomach, (– – –) stomach and upper small intestine, (· · ·) stomach and upper and middle small intestine, (–·–·–) stomach and upper, middle, and lower small intestine. Data are shown as mean ± S.E.M. ($n = 3$).

Table 2 shows the parameters obtained by fitting the percentage of the microspheres remaining verses time curves to the equations using a non-linear least square program, MILTI (Yamaoka et al., 1981). The percentage of microspheres remaining in the gastrointestinal tract is shown in Fig. 4, with the computer-fitting curves simulated using the parameters listed in Table 2. According to Fig. 4, the percentage simulated by the computer agreed with the percentage of MS-2 and MS remaining in the stomach and small intestine. Therefore, the model seems to be appropriate for explanation of our data.

The gastric emptying rate constant, $K_s$, after administering MS-2 to conscious rats was slightly smaller than that of MS. The lag time, $T_s$, in the case of MS was 0.003 h, meaning that MS was emptied from the stomach without a lag time. In contrast, $T_s$ in the case of MS-2 was 0.85 h, indicating that MS adhered strongly to the gastric mucosa and remained in the stomach for extended periods. On the contrary, the transit times of MS-2 in each segment of the small intestine, $T_u$, $T_m$, and $T_l$, were smaller than those of MS. That is, the transit of MS-2 in the small intestine was not prolonged but accelerated as compared with MS. This means that MS-2 detached from the gastric mucosa did not adhere to the small intestine at all. This is explained by the dextran derivatives leaching out of the microspheres after full hydration, and the mucin, liberated from mucosa, could interact with the polymers, preventing them from adhering to mucosal tissue again. Montisci et al. (2001) reported that the in vivo mucoadhesion of lectin-poly(lactide) microsphere conjugates depended on the balance between non-specific and specific interactions with the mucosal surfaces, and non-specific interactions were predominant compared with specific interactions in the intestine. It was probable that MS-2 detached from the gastric mucosa had a hydrophilic surface and could not interact with the mucosal surfaces contrary to MS. Consequently, the time required for 50% of MS-2 microspheres to reach the colon, $T_{50}$, was equal to that of MS. However, the time required for 50% of MS-2 microspheres to leave the stomach was 1.42 h, three times longer than that of MS. These results suggest that MS-2 is a useful dosage form targeting the gastric mucosa or prolonging gastric residence time without altering the gastrointestinal transit time.

Table 2
Rate and time constants for gastrointestinal transit of MS and MS-2

| | MS | MS-2 |
|---|---|---|
| $K_s$ ($h^{-1}$) | 0.63 | 0.53 |
| $T_s$ (h) | 0.00 | 0.85 |
| $T_u$ (h) | 0.22 | 0.02 |
| $T_m$ (h) | 0.69 | 0.14 |
| $T_l$ (h) | 1.88 | 1.72 |
| $T_u + T_m + T_l$ (h) | 2.79 | 1.87 |
| $T_{s50}$ (h) | 0.48 | 1.42 |
| $T_{50}$ (h) | 3.27 | 3.29 |

$K_s$, first-order gastric emptying rate constant; $T_s$, lag time; $T_u$, transit time through upper small intestine; $T_m$, transit time through middle small intestine; $T_l$, transit time through lower small intestine; $T_{s50}$, time required for 50% of microspheres to leave stomach; $T_{50}$, time required for 50% of microspheres to reach colon.

## 4. Discussion

This study was done to evaluate microspheres made of DS/EA mixture and CAB for oral mucoadhesive devices. In vitro evidence confirmed that microspheres containing more than 40% of DS/EA mixture adhered to the mucosal tissue significantly longer than the reference microsphere. The in vivo study revealed the gastrointestinal transit of MS-2 having a prolonged gastric emptying time. This is the first application of dextran derivatives that form polyion complexes to an oral mucoadhesive microsphere.

There are several studies for multiple-unit dosage forms with mucoadhesion to gastric mucosa, such as carbopol-dispersed microspheres (Akiyama et al., 1995), carbopol microparticles (Cuña et al., 2001), and gelatin microsphere (Wang et al., 2001). Synthetic bioadhesives, such as carbopol and polycarbophill, have been incorporated in dosage forms administered orally (Singla et al., 2000), thus many researchers apply the polymers to multiple-unit bioadhesive systems. Akiyama et al. prepared carbopol-dispersing microspheres made of polyglycerol esters of fatty acids to evaluate their mucoadhesive properties, and found that the microspheres achieved excellent distribution in the gastric cavity and had a prolonged gastric residence time. A disadvantage of carbopol, however, is its high sensitivity to the ionic environment and the change in pH (Singla et al., 2000). Their use of an ion-rich or basic pH environment may interfere with adhesion of the polymer. Thus, its sensitivity makes it difficult to control in vivo mucoadhesion. Akiyama et al. also mentioned that the mean particle size of carbopol needed to be small enough (9.43 μm) in order to swell as in the photographs shown in Fig. 1. On the other hand, the mucoadhesive properties of DS/EA mixture were not affected by ionic strength or pH in the medium (Miyazaki et al., 2002). Furthermore, DS/EA mixture powder could be used to prepare microspheres simply by sieving through a 200-mesh sieve.

In general, synthetic bioadhesives have the potential of inducing biological toxicity (Bottenberg et al., 1991). Therefore, natural polymers with bioadhesive properties were used as mucoadhesive agents for the multiple-unit dosage forms, e.g. chitosan (Shah et al., 1999) and gelatin (Wang et al., 2001). According to Shah et al. (1999), the chitosan microsphere is an effective device for stomach-specific drug delivery. Although the drug release and stability in simulated gastric fluid and permeability through gastric mucin of antibacterial agents may appear well established in vitro, the gastrointestinal transit of the chitosan microspheres is not addressed. Wang et al. (2001) developed the gelatin microspheres made of aminated gelatin in order to target the gastric mucosa. The fluorescence intensity measurement revealed the gastric retention time of aminated gelatin was about 50% remaining, 2 h after administration to rats. However, they did not mention that the gelatin microspheres maintained an intact shape and were still capable of retaining the incorporated drug. On the other hand, the microspheres used in our study were found in the gastrointestinal tract with an intact shape for up to 5 h.

In the mucoadhesion process, it is necessary for swelling and expansion of the polymer chain since interpenetration and entanglement of the polymers and the mucous networks are considered to be responsible for adhesion (Duchêne et al., 1988). Therefore, bioadhesives should swell and expand rapidly when they come in contact with water. On the other hand, over-swelling causes polymer molecules to separate from the microsphere resulting in decreased mucoadhesion. Thus, the adhesive force of natural polymers, in general, was comparatively low among various bioadhesives (Jackson and Perkins, 2001). In order to overcome this discrepancy, chemical cross-linking procedures (e.g. ephiclorohidrin, glutaraldehyde) giving rise to the formation of non-soluble networks, have been considered. For example, the preparation of the aminated gelatin microspheres mentioned above was conducted by a chemical cross-linking procedure. However, it is not desirable because the use of cross-linkers can lead to toxic side effects, due to the presence of residual cross-linking agents or to an unwanted reaction between drug and cross-linker. So, we used a polyion complex formation as a physical cross-linking method. Although dextran derivatives are also natural polymers with high solubility in water, polyion complex formation prevents rapid dissolution of the polymers and allows the polymers to remain in the microsphere.

Further studies to confirm the utility of this microsphere for gastric drug delivery are required. Several reports have shown that gastric adhesion could significantly improve the absorption of drugs such

as furosemide and riboflavin (Akiyama et al., 1998), and the gastric residence of drugs such as antibiotics against *Helicobacter pylori* (Nagahara et al., 1998). These are potential topics for future studies.

## 5. Conclusion

The mixtures of opposite-charged dextran derivatives were found to be useful adhesive agents for multiple-unit mucoadhesive devises. Microspheres containing more than 40% of EA/DS mixture showed good mucoadhesive performance in vitro. Furthermore, microspheres containing 40% of EA/DS strongly adhered to the gastric mucosa and remained in the stomach after administration to rats. The microspheres are expected to be applied to gastric-targeted preparation or drug delivery systems, which have a narrow absorption window in the upper intestine.

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