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Chitosan dispersed system for colon-specific drug delivery

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Abstract

A chitosan dispersed system (CDS), which was composed of active ingredient reservoir and the outer drug release-regulating layer dispersing chitosan powder in hydrophobic polymer, was newly developed for colon-specific drug delivery. An aminoalkyl methacrylate copolymer RS (Eudragit® RS) was selected as a hydrophobic polymer because it is hardly dissolved in acidic medium in which easily dissolves chitosan. In order to obtain the bi-functional releasing characteristics, i.e. time dependent and site specific, capsules containing the active ingredient (Drug Capsules) were coated by the chitosan dispersed hydrophobic polymer, resulting in CDS Capsules. The release rate could be controlled by changing the thickness of the layer. Furthermore, for colon-specific drug delivery, an additional outer enteric coating was necessary to prevent the drug release from CDS Capsules in the stomach, since chitosan dispersed in the layer dissolves easily under acidic conditions. Resultant enteric-coated CDS Capsules reached the large intestine within 1–3 h after oral administration and they were degraded at the colon in beagle dogs. © 2002 Published by Elsevier Science B.V.

Keywords: Chitosan dispersed system; Chitosan powder; Capsule; Colon-specific; Drug delivery

1. Introduction

Within recent years, there is increasing interest in specific delivery of drugs to the colon via the oral route, because there are useful technologies for treating colon-specific diseases, that is, inflammatory disease including Crohn's disease and ulcerative colitis (Hanauer, 1996). Anatomical and physiological features of the colon was reviewed by Watts and Illum, 1997.

The major function of the colon is to absorb water and electrolytes (each day up to 2000 ml of fluid enters into the colon through the ileocecal valve). Although the absorption capacity in human colon is lower than that in the small intestine (surface area is 0.3, 120 m^2 respectively), the residence time of the preparations in human colon is 2–3 days. This long colonic residence time provides a significant opportunity for the absorption of drugs (Edwards, 1997). Furthermore, the colon is considered as an important part for administrating the peptide drugs for systemic

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therapy, because the hydrolytic enzyme activity in the colon is lower than that in the small intestine (Saffran et al., 1986; Bai and Chang, 1993; Bernkop-Schnurch, 2000). The majority of materials in the human colon ($\sim$220 g of wet materials, equivalent to 35 g of dry matter) is bacteria in the range of 10^{11}–10^{12} CFU/ml consisting over 400 species of bacteria, predominantly anaerobes, and a small number of fungi (Watts and Illum, 1997). In case of humans, the highest pH levels (7.5 ± 0.5) are found in the terminal ileum. On the entry into the colon, the pH drops to 6.4 ± 0.6. The pH in the mid-colon is 6.6 ± 0.8 and the pH in the left-colon is 7.0 ± 0.7. The fall in pH on entry into the colon is due to the presence of short chain fatty acids arising from the bacterial fermentation of polysaccharides. Colonic pH goes down to 4.7 ± 0.7 in a group of untreated ulcerative colitis disease, whereas to 5.5 ± 0.4 in a group of treated (Watts and Illum, 1997).

Site-specific targeting of drugs to the colon has been tried by several different approaches, i.e. pH control (Yamada et al., 1995; Khan et al., 2000; Ashford et al., 1993), time-control (Steed et al., 1997; Niwa et al., 1995; Ueda et al., 1994), pressure-control (Hu et al., 1998; Takaya et al., 1998; Muraoka et al., 1998; Jeong et al., 2001; Fukui et al., 2000), pro-drug and colon-specific polymer (Tozaki et al., 1997; Kakoulides et al., 1998).

There are many polysaccharides which have been tried as colon-specific systems, from algal origin (e.g. alginates), plant origin (e.g. pectin (Semdé et al., 1998 Ahrabi et al., 2000 Semdé et al., 2000), guar gum, amylose (Siew et al., 2000; Milojevic et al., 1996a,b), microbial origin (e.g. dextran, xanthan gum) and animal origin (chitosan, chondroitin).

Chitosan is completely digested by the colonic bacteria and is toxicologically harmless material of low cost and turned out to be a useful excipient in various pharmaceutical formulations (Dodane and Vilivalam, 1998). Chitosan has been used for colon-specific drug delivery as the forms of microspheres (Lorenzo-Lamosa et al., 1998) and capsules (Tozaki et al., 1997) because of its biodegradable properties by colonic bacteria. Under the presence of rat cecal contents in the dissolution medium, chitosan is degraded. It is also suggested that the enzymes, which are produced for the degradation of chitosan decrease the pH of the cecal contents and chitosan is dissolved.

Since chitosan owns a number of amine groups, it dissolves in weak acidic conditions, but is insoluble in higher pH conditions due to the deprotonation of the amines (Ravindra et al., 1998). Chitosan is hardly dissolved in organic solvents, in which the water-insoluble polymers are dissolved.

We have been developing a novel colon-specific drug delivery system using chitosan powder. We named this system 'chitosan dispersed system (CDS)'. This system has the release-regulating layer composed of the mixture of water-insoluble polymer and chitosan powder. This mixed layer can improve the release properties of the film compared with the ordinary sustained release preparations. In the case of using chitosan as a coating material, we have to dissolve it in a weak acid such as acetic acid. But, by dispersing chitosan powder in the water-insoluble polymer solution, we do not have to use acetic acid and can control the drug release time-dependently and site-specifically. The permeability of the drug to the layer is able to control by the thickness of the layer.

As chitosan dissolves in acidic conditions, outer layer has to be coated by the enteric-polymer in order to pass through the stomach intact (for colon-specific drug delivery). In this study, single-unit type preparations using the CDS was selected to ascertain the degradation characteristics in the gastrointestinal tract. As a model drug, acetaminophen (AAP) was used, because AAP is known to be well absorbed from the wide regions of the intestinal tract (Yamada et al., 1995).

2. Experimental

2.1. Materials

AAP was purchased from Iwaki seiyaku Co., Ltd, Japan. Other excipients used are as follows: a seed core; purified sucrose spheres (Nonpareil® 103, Freund Industrial Co., Ltd, Japan), a binder;

hydroxypropylmethylcellulose (TC-5E, Shin-Etsu Chemical Co., Ltd, Japan), a capsule; size two hard gelatin capsule (Shionogi Qualicaps Co., Ltd, Japan), a release-regulating layer; Eudragit® RS (Röhm Pharma, Germany) and chitosan (FLONAC® C, low molecular weight chitosan, deacetylation degree 91.6%, Kyowa Technos Co., Ltd, Japan), an enteric-polymer; methacrylic acid–ethyl acrylate copolymer (Eudragit® L 100-55, Röhm Pharma, Germany), an anti-static agent; magnesium stearate (Mg-St, Taihei Chemical Industrial Co., Ltd) and talc (Nippon Talc. Co., Ltd). All other reagents and solvents were of analytical grade.

2.2. Particle size distributions

Particle size was measured by Laser Diffraction Particle Size Distribution Analyzer (HELOS & RODOS, Sympatec GmbH, Germany).

2.3. Preparation of Drug Cores

Drug Cores were prepared by layering AAP powder (900 g) with a binder on the surface of Nonpareil 103 (1450 g) using a Centrifugal Fluidizing Granulator (CF-Granulator, CF-360, Freund Industrial Co., Ltd, Japan). TC-5E ethanol (95 v/v %) solution (1.5 w/w %) was used as a binder (Total usage, 23.4 g). Operating conditions were as follows: rotating speed, (250 r.p.m.), inlet air temperature, (40 °C), inlet air volume, (150 l/min), spray air volume, (10 l/min) and binder flow rate, (15.3 g/min).

2.4. Preparation of Drug Capsules

Drug Capsules were prepared by filling Drug Cores (300 mg) in size two capsules by using a Full-Automatic Capsule Filling Machine (LIQFIL super 40, Shionogi Qualicaps Co., Ltd, Japan).

2.5. Preparation of CDS capsules

The coating was performed dropping the suspension (4 w/w % Eudragit® RS 95 w/w % ethanol solution (300 g (12 g as a solid))/chitosan powder (24 g)) onto the Drug Capsules (500 g) in a CF-Granulator. Operating conditions were as follows: rotating speed, (250 r.p.m.), inlet air temperature, (30 °C), inlet air volume, (150 l/min), liquid suspension dropping rate, (3.5 g/min).

2.6. Preparation of enteric-coated CDS capsules

2.6.1. Enteric coating of Drug Capsules

Enteric layer's components (Eudragit® L100-55/Mg-St/95% ethanol = 5/4/95) were coated on the surface of Drug Capsules (100 g) by using a CF-Granulator. Operating conditions are as follows: rotating speed, (250 r.p.m.), inlet air temperature, (30 °C), inlet air volume, (150 l/min), spray air volume, (12 l/min), liquid solution flow rate, (4.3 g/min).

2.6.2. Enteric coating of CDS capsules

With dropping the suspension (3.5 w/w % Eudragit® L100-55 95 w/w % ethanol solution/talc = 150 g/5.25 g) onto the CDS Capsules (100 g), coating was performed by using a CF-Granulator. Operating conditions were as follows: rotating speed, (250 r.p.m.), inlet air temperature, (30 °C), inlet air volume, (150 l/min), liquid suspension dropping rate, (3.5 g/min).

2.7. In vitro release testing

Release testing was carried out by the Japanese Pharmacopoeia (JP XIV) Paddle method (100 r.p.m.) using a sinker or basket method (100 r.p.m.) for evaluation of drug release. Three kinds of fluids, i.e. JP 1st fluid simulating a gastric juice (pH 1.2), JP 2nd fluid simulating an intestinal fluid and pH 4.0 acetate buffer solution simulating the colon fluid were used as the dissolution medium. AAP concentrations in the dissolution medium were spectrophotometrically determined.

2.8. In vivo testing

Twenty 2-year-old male beagle dogs weighing between 13 and 15 kg were fasted for 18 h prior to the oral administration. Five enteric-coated CDS (E-CDS) Capsules (40 mg/dog as AAP) were administered with 50 ml of water.

2.8.1. Gastrointestinal transit of E-CDS Capsules

Beagle dogs were killed at the designated time (1, 2, 3, 4 and 6 h after the administration ($n = 3$ (each time)) and the capsules were collected from the gastrointestinal tracts.

2.8.2. In vivo release study

Blood samples (5 ml) were collected at designated time intervals. AAP plasma concentrations were determined by HPLC method with an internal standard of 2-acetaminophenol. The pH 3.0 phosphate buffer/acetonitrile/methanol (10/1/0.5 v/v %) was used as a mobile phase at a flow rate of 1.0 ml/min, detection wavelength was 247 nm. HPLC was performed by ODS column (YMC-Pack ODS-A (150 × 6.0 mm I.D.), YMC Co., Ltd, Kyoto, Japan) at 40 °C.

3. Results and discussion

3.1. Fundamental structure of CDS Capsule

The basic structure of CDS Capsule is schematically shown in Fig. 1. Capsules containing the Drug Cores (Drug Capsules) were coated with the chitosan dispersed hydrophobic polymer. Hydrophobic polymers are hardly dissolved in acidic medium in which easily dissolve chitosan. In order to obtain the bi-functional releasing characteristics, i.e. time dependent and site specific, Drug Capsules should be coated by the chitosan dispersed hydrophobic polymer. Especially for colon-specific drug delivery, an additional outer enteric coating is necessary to prevent drug release from CDS Capsules in the stomach, since the dispersed chitosan easily dissolves under acidic conditions. CDS Capsules with this outer enteric film (E-CDS Capsules) can exhibit the releasing characteristics for colon-specific drug delivery. The gastrointestinal transit of pharmaceutical dosage forms was reported by Davis et al. (1986). In general, the average of the small intestinal transit time is known to be about 3 h in humans. In this study, as the beagle dogs were used for in vivo release study, the drug releasing lag-time after emptying from the stomach was settled 2 h by the appropriate thickness of chitosan dispersed layer (Yamada et al., 1995).

Since coarse chitosan powder was not successfully coated on the surface of capsules, therefore the fine chitosan powder (FLONAC® C 100M, 100 mesh 80% pass, mean particle size 94.88 μm) was used. The surface of CDS Capsule was a little bit rough, because the particle size of chitosan powder was still a little bit larger for getting the smooth surface.

3.2. Effect of chitosan dispersed water-insoluble polymer layer thickness on drug release from CDS Capsules

Fig. 2 shows the release profiles of AAP from the CDS Capsules in 1st fluid (pH 1.2) and in 2nd fluid (pH 6.8). As the thickness of the layer became increase, the drug release rate became decrease. It was found that Eudragit® RS was preferred for obtaining the predetermined lag-time (the time the drug is not released). The lag-time could be controlled by the thickness of the release-regulat-

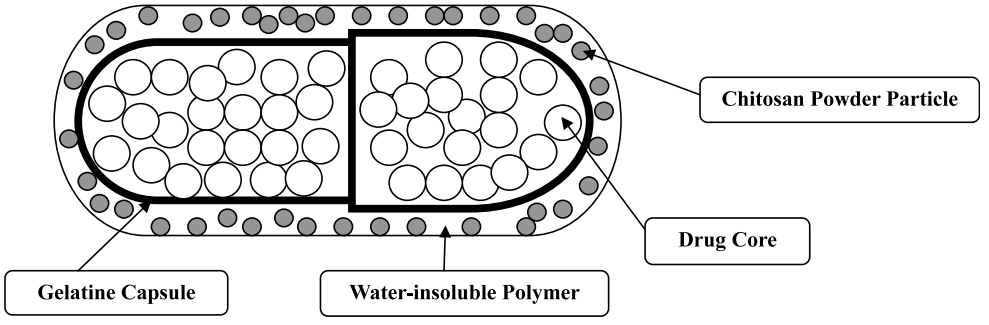

Fig. 1. Fundamental structure of CDS Capsule.

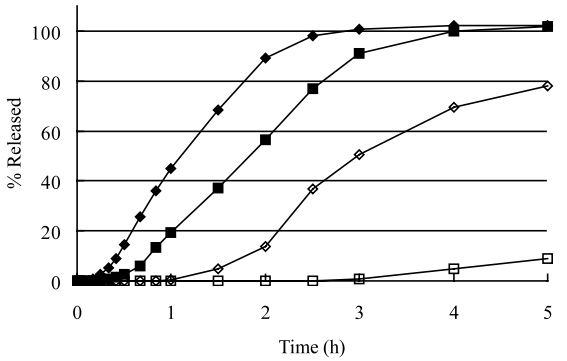

Fig. 2. Release profiles of AAP from CDS Capsules (1st or 2nd fluid 900 ml, 37 °C, Paddle method, 100 r.p.m.) (Each value represents the mean ($n = 3$)). Eudragit RS/Chitosan = 4/8 (9 mg coating as a solid per Drug Capsule) (pH 1.2) (◆). Eudragit RS/Chitosan = 8/16 (18 mg coating as a solid per Drug Capsule) (pH 1.2) (■). Eudragit RS/Chitosan = 4/8 (9 mg coating as a solid per Drug Capsule) (pH 6.8) (◇). Eudragit RS/Chitosan = 8/16 (18 mg coating as a solid per Drug Capsule) (pH 6.8) (□).

ing layer (The mixture layer of Eudragit® RS and chitosan powder).

3.3. Effect of the pH on drug release from CDS Capsules

Fig. 3 shows the release profiles of AAP from CDS Capsules in 2nd fluid, 1st fluid and pH 4.0 solution, respectively. Drug release lag-time in 2nd fluid was about 2 h by the appropriate amount of chitosan dispersed layer (Eudragit® RS/chitosan powder = 12 g/24 g as a solid per 500 g Drug Capsules, 26 mg (chitosan dispersed layer amount as a solid)/Drug Capsule). As described above, chitosan dissolves in acidic conditions, then the drug release rate in acidic conditions is faster than that in 2nd fluid. The permeability of the drug to the layer was dependent on the thickness of the layer. Therefore, the releasing site in the gastrointestinal tract could be controlled by adjusting the layer thickness.

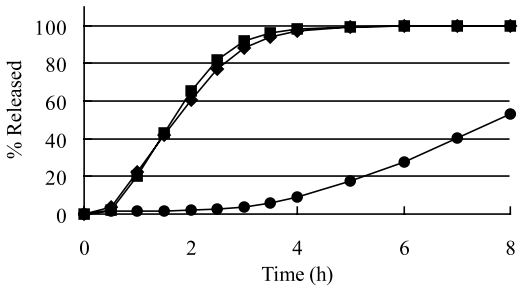

Fig. 3. Release profiles of AAP from CDS Capsules (1st, pH 4.0 or 2nd fluid 900 ml, 37 °C, Paddle method, 100 r.p.m.) (Each value represents the mean ($n = 3$)). 1st fluid (◆); pH 4.0 (■); 2nd fluid (●).

3.4. Enteric-coating

In order to pass through the stomach intact, CDS Capsules have to be coated with an enteric polymer. Before coating the enteric layer on the surface of CDS Capsules, acid-resistibility was checked using Drug Capsules. The acid-resistibility of the enteric-layer could be checked clearly.

3.4.1. Effect of the thickness of the enteric layer on the acid-resistibility using Drug Capsules (enteric coating of Drug Capsules)

Fig. 4 shows the release profiles of AAP from the various coating level of enteric capsules in 1st fluid. Lag-time increases with the coating level increases. Even if the enteric coated capsule with 4 h lag-time (240 mg (enteric coating amount as a

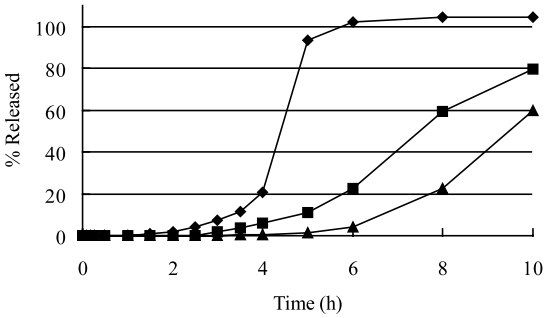

Fig. 4. Release profiles of AAP from E-Drug Capsules (1st fluid 900 ml, 37 °C, Paddle method, 100 r.p.m.) (Each value represents the mean ($n = 3$)). (◆) 1 h, enteric coating was performed for 1 h (80 mg enteric polymer was coated per capsule). (■) 2 h, enteric coating was performed for 2 h (160 mg enteric polymer was coated per capsule). (▲) 3 h, enteric coating was performed for 3 h (240 mg enteric polymer was coated per capsule).

solid)/Drug Capsule), AAP was released within a few minutes in 2nd fluid. This was because that the enteric polymer (Eudragit® L100-55) dissolves quickly in 2nd fluid.

3.4.2. Enteric coating of CDS Capsules

Mean gastric emptying time in humans is well known to be about 2 h (Davis et al., 1986). On the other hand, in case of beagle dogs, gastric emptying time is known to be about 0.3 h (Murata et al., 1998). Therefore, in this study, the drug releasing lag-time after the administration was made more than 1 h. But, the gastric emptying time is unpredictable because the individual difference is very big. As a result, E-CDS Capsules having 10 h of lag-time in 1st fluid (the drug was not released from the preparation in the stomach) were selected for the in vivo evaluations (223 mg (enteric coating amount as a solid)/CDS Capsule). Even if the acid-resistibility in 1st fluid is more than 10 h, the enteric polymer dissolves promptly in 2nd fluid as mentioned above.

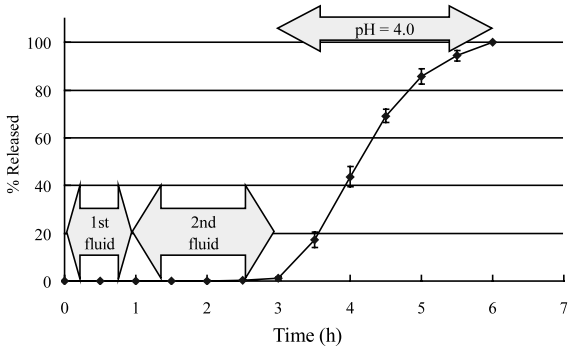

Fig. 5. Release profile of AAP from E-CDS Capsule (900 ml, 37 °C, Rotating basket method, 100 r.p.m.) (Each value represents the mean ± SE of five data).

3.5. In vitro evaluation of E-CDS Capsules under simulating the gastro-intestinal tracts

The release tests were carried out in 1st fluid for 1 h, immediately followed by 2nd fluid for 2 h, i.e. under simulated gastrointestinal conditions of pH and transit times in beagle dogs as shown in Fig. 5. The basket method was adopted for the release tests not to damage a sample in liquid exchange.

Table 1
Gastrointestinal transit of E-CDS capsules after oral administration of five capsules to each beagle dog ($n = 3$)

	Dog No.	Stomach	Duodenum	Jejunum	Ileum	Colon	Total
1 h	1			1	4		5
	2				5		5
	3	5					5
2 h	4	2		3			5
	5				4		4
	6			1	3	1	5
3 h	7				1	4(3)	5
	8				5(1)		5
	9				5		5
4 h	10						0
	11						0
	12						0
6 h	13						0
	14						0
	15						0

Each number represents the capsule number which were collected from the gastrointestinal tract of beagle dogs. The number in the parentheses represents the capsule number which were degraded in the gastrointestinal tract of beagle dogs. In case of 4 and 6 h, after oral administration, capsules were not collected because they were already degraded and could not be counted.

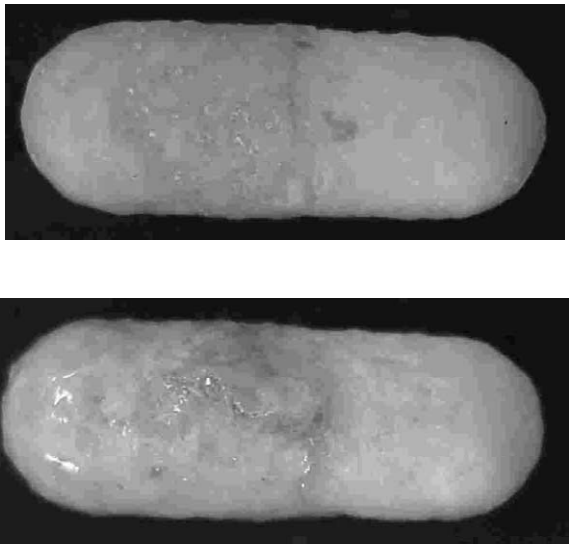

Fig. 6. Photographs of capsules, which were collected from the stomach at 2 h after the administration (dog No. 4).

No drug release from E-CDS Capsules was observed in an artificial gastric juice (1st fluid) for more than 1 h, and after replacement of the medium to an artificial intestinal fluid (2nd fluid), the drug was not practically released in the following 2 h. The results show that the E-CDS Capsules are able to pass intact through the stomach and the small intestine. After replacement of the medium to a pH 4.0 buffer solution, in which chitosan powder were easily dissolved, the release of drug markedly increased.

It was recently reported that the pH in the colon is actually lower than that of the small intestine by the acidification of colonic contents by the products of bacterial fermentation (Tozaki et al.,

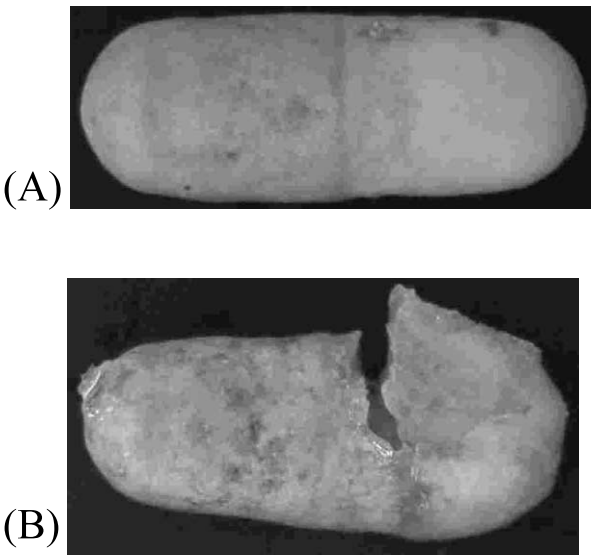

Fig. 7. Photographs of capsules, which were collected from the ileum at 3 h after the administration. (A) Capsule which was not degraded in the ileum (dog No. 7), (B) capsule which was degraded in the ileum (dog No. 8).

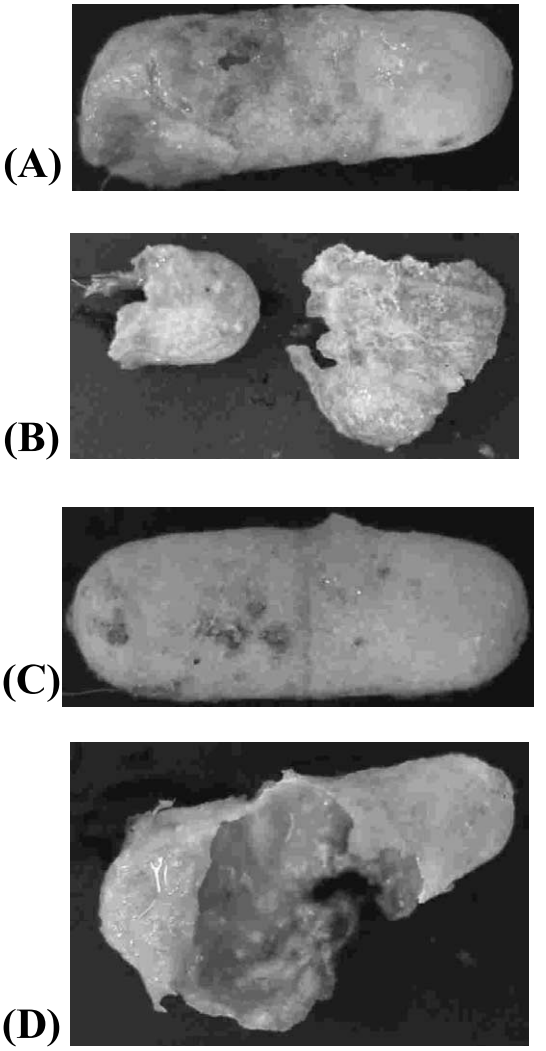

Fig. 8. Photographs of capsules, which were collected from the colon at 3 h after the administration. (A), (B) and (D) capsule which was degraded in the colon (dog No. 7). (C) Capsule which was not degraded in the colon (dog No. 7).

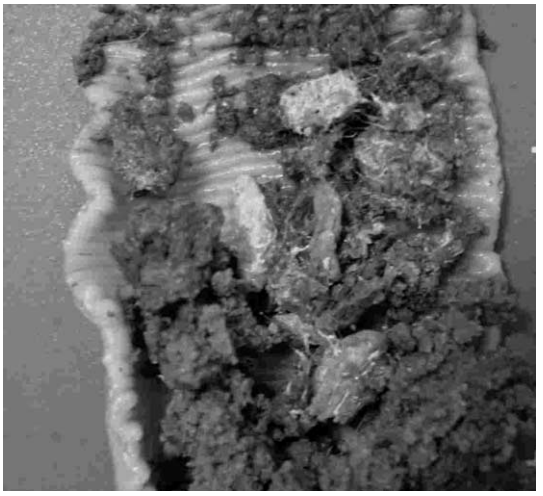

Fig. 9. Photographs of degraded capsules, which were in the colon at 4 h after the administration. (dog No. 11).

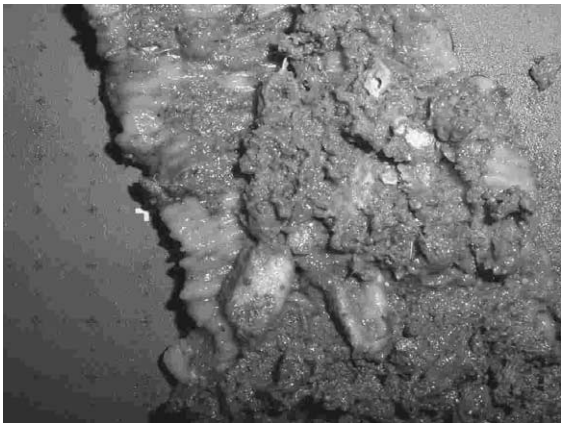

Fig. 10. Photographs of degraded capsules, which were in the colon at 6 h after the administration. (dog No. 13).

1997; Pye et al., 1990). In case of severe inflammatory bowl disease, the colonic pH often drops to between 1 and 5 (Evans et al., 1988). Therefore, from a point of simplification of the evaluation, pH 4.0 standard buffer solution which is widely used for the evaluation of the drug release from the preparations was used in this study.

E-CDS Capsules would be able to pass intact through the stomach and the small intestine, and the drug would be released immediately at the colon.

3.6. In vivo evaluation of E-CDS Capsules

3.6.1. Gastrointestinal transit of E-CDS Capsules

Although single-unit type preparations were selected to ascertain the degradation characteristics, for minimizing the gastrointestinal transit time differences between capsules, five E-CDS Capsules were administered to beagle dogs. The gastrointestinal transit of E-CDS Capsules after oral administration is shown in Table 1. After oral administration of five E-CDS Capsules to beagle dogs, they were emptied from the stomach at least within 2 h. The capsules which were found in the stomach were not degraded within 2 h after the administration (Fig. 6). After 3 h, almost of all the capsules moved into the ileum or into the colon (Table 1). Only one capsule (Fig. 7 (B)) was degraded within the capsules which were found in the ileum (dog No. 8, Table 1). Almost of all the capsules which were found in the ileum were not degraded (Fig. 7 (A)). Capsules existing in the colon were almost degraded (Fig. 8). That is, three capsules were degraded in four capsules which were found in the colon (dog No. 7, Table 1). After 4 and 6 h, un-degraded capsules were not found in the gastrointestinal tracts (Table 1), but uncountable degraded capsules were found in the colon (Figs. 9 and 10). These results suggest that E-CDS Capsules were mainly reached the large intestine within 2–4 h after the oral administration and they were degraded in the colon in beagle dogs within 3–4 h after the administration.

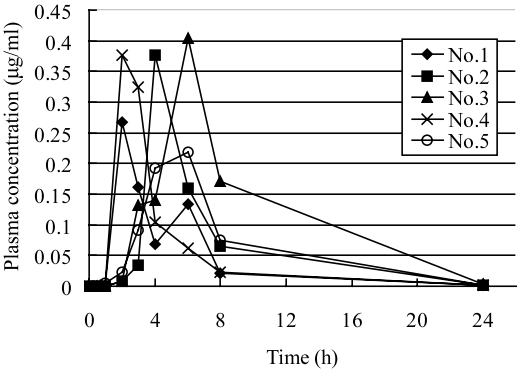

Fig. 11. Plasma concentration vs. time curves of AAP after the administration of five E-CDS capsules to beagle dogs ($n = 5$).

Table 2
Pharmacokinetic parameters of AAP in beagle dogs ($n = 5$)

Preparation	T_{max} (h)	C_{max} (μg/ml)	AUC (μg h/ml)	MRT (h)
E-CDS Capsule	4.00±0.82	0.34±0.04	1.60±0.27	5.71±0.49

Each value represents the mean±SE of 5 dogs.

3.6.2. In vivo release study

Fig. 11 shows the plasma concentration-time curve of AAP after the administration of five E-CDS Capsules to beagle dogs ($n = 5$). Pharmacokinetic parameters are listed in Table 2. Listed values are as follows; maximum plasma concentration (C_{max}), time to peak concentration (T_{max}), area under the concentration-time curve (AUC), mean residence time (MRT). In some dogs, the drug concentration in the plasma was increasing at the early time after the administration. The pH in the stomach decreased when the capsules were administered (Akimoto et al., 2000). Therefore, in this study, the pH in the stomach of almost of all the beagle dogs would be acidic conditions. Considering the results of the transit testing, it was suggested that the E-CDS capsules were degraded in the colon within 1–4 h after the administration and drugs were absorbed from the colon. It was found that the E-CDS Capsules would be useful for treating the colonic diseases.

4. Conclusion

A CDS for colon-specific drug delivery was newly developed. In order to obtain the bi-functional releasing characteristics, i.e. time dependent and site specific, Drug Capsules were coated with chitosan dispersed layer. Chitosan dispersed layer could be coated on the surface of size two hard gelatin capsules containing active ingredients, and the release rate could be controlled by changing the thickness of the chitosan dispersed layer. For protecting the drug release from the preparations in the stomach, CDS Capsules were further coated with an enteric polymer. Resultant E-CDS Capsules reached the large intestine within 1–3 h after oral administration and they were degraded in the colon. E-CDS Capsules were found to be a useful drug delivery capsule to the colon, because they were degraded in those regions of beagle dogs.

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