Multiparticulate Chitosan-Dispersed System for Drug Delivery

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A multiparticulate chitosan-dispersed system (CDS), which is composed of the drug reservoir and the drug release-regulating layer, was developed for drug delivery. The drug release-regulating layer is a mixture of water-insoluble polymer and chitosan powder. The drug is released from CDS pellets in all regions of the gastrointestinal tract (from the stomach to the colon). CDS pellets containing chitosan powder were designed to dissolve chitosan powder partly in the release-regulating layer in the stomach and release part of drug. After passing through the stomach, the drug is released from CDS pellets at a constant speed in the small intestine. In the large intestine, CDS pellets were designed to disintegrate the remaining chitosan powder at an accelerated speed and the remaining drug in CDS pellets is released. The drug release rate can be controlled with the thickness of the chitosan-dispersed water-insoluble layer. Furthermore, for colon-specific drug delivery, an additional outer enteric coating is necessary to prevent drug release from CDS pellets in the stomach, because the chitosan-dispersed water-insoluble layer dissolves gradually under acidic conditions. The resulting enteric-coated CDS (E-CDS) pellets were found to permit colon-specific drug delivery. In this study, the multiparticulate CDS was adopted not only for colon-specific drug delivery but also for sustained drug release.

Key words chitosan-dispersed system; chitosan powder; multiparticulate; sustained release; colon-specific delivery

Multiparticulate systems have several advantages in comparison to the conventional single unit for controlled-release technology, such as more predictable gastric emptying and fewer localized adverse effects than those of single-unit tablets or capsules.[1,2] Chitosan is a biodegradable, toxicologically harmless, low-cost material that is a useful excipient in various pharmaceutical formulations.[3—5] Chitosan has been used for sustained-release preparations because of its low solubility in the small intestine.[6—8] Chitosan has mucoadhesive properties[9] and it is also used for transdermal drug delivery systems.[10] Furthermore, chitosan is known to be an absorption enhancer.[11,12] Chitosan has also been used for colon-specific drug delivery as microspheres[13] and capsules[14] because it is biodegradable by colonic bacteria. Multiple-unit systems have a larger surface area than single-unit systems, leading to more rapid release of drug in the colon. In the above examples, chitosan was coated after dissolution in weak acid such as acetic acid, resulting in the problem of acid residue in the preparations.

As previously reported,[15] we developed a novel colon-specific drug delivery system using chitosan powder called the chitosan-dispersed system (CDS). This release-regulating layer is composed of a mixture of water-insoluble polymer and chitosan powder. By dispersing chitosan powder in water-insoluble polymer solution, drug release can be controlled time dependently and site specifically. Chitosan-dispersed in water-insoluble polymer dissolves gradually in the stomach and rapidly in the colon. The system reported was a single-unit system[15] used for only colon-specific drug delivery. The present study was designed to develop a CDS available not only for colon-specific drug delivery but also for sustained drug delivery using a multiple-unit CDS.

Experimental

Materials Acetaminophen (AAP, Iwaki Seiyaku Co., Ltd., Japan) was used as a model drug. Other excipients used were as follows: the seed core was purified sucrose spheres (Nonpareil 103, Freund Industrial, Co., Ltd., Japan); the binder was hydroxypropylmethylcellulose (TC-5E, Shin-Etsu Chemicals, Japan); the release-regulating layer was Eudragit RS (Röhm Pharma, Germany); chitosan (FLONAC C, low molecular-weight chitosan, deacetylation degree 91.6%, Kyowa Technos Co., Ltd., Japan); the enteric-polymer was Eudragit L100-55 (Röhm Pharma, Germany); and the antistatic agent was magnesium stearate (Mg-St, Taihei Chemical Industrial Co., Ltd., Japan). All other reagents and solvents were of analytical grade.

Particle Size Distribution The particle size of chitosan powder was measured with a laser diffraction particle size distribution analyzer (Helos & Rodos, Sympatec GmbH, Germany).

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). TC-5E ethanol solution (1.5 w/w%) was used as a binder. The operating conditions were: rotating speed, 250 rpm; inlet air temperature, 40 °C; inlet air volume, 150 l/min; spray air volume, 10 l/min; binder flow rate, 15.3 g/min; and amount of binder used, 23 g as a solid.

Preparation of Enteric-Coated Drug Cores Enteric-coated drug cores (E-Drug) were prepared to remain intact in the stomach and then to release the active ingredient in the upper small intestine. Enteric components (Eudragit L100-55/Mg-St/95% ethanol=5/4/95) (84 g as a solid) were coated on the surface of drug cores (360 g) using a CF-granulator. The operating conditions were: rotating speed, 250 rpm; inlet air temperature, 30 °C; inlet air volume, 150 l/min; spray air volume, 12 l/min; and liquid flow rate, 4.3 g/min.

Preparation of Sustained-Release Pellets Eudragit RS ethanol solution (4.0 w/w%) (24 g as a solid) was coated onto drug cores (360 g) using a CF-granulator. The operating conditions were: rotating speed, 250 rpm; inlet air temperature, 30 °C; inlet air volume, 150 l/min; spray air volume, 12 l/min; and liquid solution flow rate, 4.6 g/min.

Preparation of CDS Pellets With spraying of 4.0 w/w% Eudragit RS ethanol solution, chitosan powder was added and coated onto drug cores using a CF-granulator. The ratio of Eudragit RS to chitosan powder was 1 to 2. Chitosan powder (100 M, 100 mesh 80% pass, mean particle size 84.31 μm) was used for powder coating. The operating conditions were: rotating speed, 250 rpm; inlet air temperature, 30 °C; inlet air volume, 150 l/min; spray air volume, 12 l/min; and liquid solution flow rate, 4.6 g/min. Samples at various coating levels were taken. CDS-2 h, CDS-4 h, and CDS-7 h represent the coating times of 2 h, 4 h and 7 h, when chitosan-dispersed layer (chitosan/Eudragit RS=1/2 as a solid) of 72 g, 144 g, and 252 g were coated per 360 g of drug core, respectively.

Preparation of E-CDS Pellets Enteric components (Eudragit L100-

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55/Mg-St/95% ethanol=5/4/95) (84 g as a solid) were layered on the surface of CDS-7 h pellets (360 g). The operating conditions were the same as for the preparation of CDS pellets, except that the liquid flow rate was 4.3 g/min.

Morphological Examination The surface of pellets and chitosan powder were examined under a scanning electron microscope (SEM, JSM-T20, JEOL Ltd., Japan). Mean diameters of pellets were calculated based on the area of pellets ($n=10$). Areas of pellets were obtained by morphological observation under an optical microscope (Digital HD Microscope VH-7000, KEYENCE, Japan).

***In Vitro* Release Testing** Release testing (300 mg as AAP) was carried out in accordance with the *Japanese Pharmacopoeia* (JP XIV) rotating basket method (100 rpm). Three fluids, *i.e.*, JP 1st fluid simulating gastric juice (pH 1.2 aqueous solution), JP 2nd fluid simulating intestinal fluid (pH 6.8 phosphate buffer), and pH 4.0 acetate buffer, in which chitosan powder was easily dissolved, were used as dissolution media. Concentrations of AAP in the dissolution media were determined spectrophotometrically (detection wavelength, 247 nm).

***In Vitro* Degradation of CDS Pellets in Rat Cecal Contents** Fresh rat cecal contents (18 g) were suspended in the 2nd fluid (250 ml) and divided into two groups. One was heat-treated at 80 °C for 30 min and the other was not heat-treated. CDS pellets were added to the two group liquids. Each liquid was stirred for 4 h at 37 °C under aerobic conditions.

***In Vivo* Testing** Male Wistar rats weighing 240—260 g were fasted for 24 h prior to oral administration of AAP solution (4 mg/ml, 10 mg/kg rat body weight as AAP) or pellets (10 mg/kg rat body weight as AAP) with 0.5 ml of water. Blood samples (0.25 ml) were collected from the jugular vein using heparinized syringes at designated time intervals. AAP plasma concentrations were determined by the modified HPLC method[16] with an internal standard of 2-acetaminophenol. A mixture of pH 3.0 phosphate buffer/acetonitrile/methanol (10/1/0.5 v/v) was used as the mobile phase at a flow rate of 1.0 ml/min. HPLC was performed on an ODS C_{18} column (150 mm×6.0 mm) kept at 40 °C with an ultraviolet detector (detection wavelength, 247 nm).

Time to peak concentration (T_{max}), maximum plasma concentration (C_{max}), area under the plasma concentration–time curve (*AUC*), and mean residence time (*MRT*) were calculated with the noncompartment method. To compare bioavailability between different preparations, Student's *t*-tests, were performed using the SAS statistical analysis software package (SAS Institute, Cary, NC, U.S.A.). A probability level of $p<0.05$ was considered statistically significant.

Results and Discussion

Fundamental Structure of CDS Pellets The basic structure of CDS pellets is schematically shown in Fig. 1. The drug core containing the active ingredient was coated with chitosan-dispersed water-insoluble polymer. Water-insoluble polymers such as Eudragit RS are difficult to dissolve in acidic medium in which chitosan dissolves. To obtain the bi-functional release characteristics, *i.e.*, time dependent and sitespecific, drug cores should be coated with chitosan-dispersed water-insoluble polymer. By adjusting the layer characteristics, the release site of drug in the gastrointestinal tract may be controlled. Especially for colon-specific drug delivery, an additional outer enteric coating is necessary to prevent the drug release from CDS pellets in the stomach, since the dispersed chitosan gradually dissolves under acidic conditions. CDS pellets with this outer enteric film (enteric-coated CDS pellets, E-CDS pellets) could exhibit release characteristics for colon-specific drug delivery.

Morphological Examination Figure 2 shows scanning electron microphotographs of chitosan powder particles (A), seed core (B), drug core (C), E-drug (D), and representative CDS pellet (E) and E-CDS pellet (F). The diameters of these pellets were around 1.0 mm.

Sustained Drug Release Using CDS Pellets CDS pellets were prepared for sustained drug release and *in vitro* and *in vivo* evaluations were carried out.

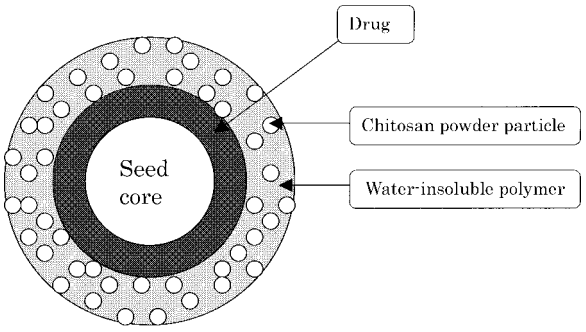

Fig. 1. Structure of CDS Pellets

(1) Dissolution Profiles of CDS Pellets: Figure 3A shows release profiles of AAP from CDS pellets (CDS-2 h, CDS-4 h and CDS-7 h) or SR pellets in artificial intestinal fluid (2nd fluid). Diameters [mean±S.D. ($n=10$)] of drug core, CDS-2 h pellets, CDS-4 h pellets and CDS-7 h pellets were 1062±31 μm, 1128±48 μm, 1178±55 μm and 1338±27 μm, respectively. As a results, the thickness of the chitosan-dispersed layer of CDS-2 h pellet, CDS-4 h pellet and CDS-7 h pellet were about 33 μm, 58 μm, and 138 μm, respectively. The release rate of AAP decreased with the increasing thickness of the layer. These results indicate that the drug release rate could be controlled by the thickness of the chitosan-dispersed layer in the gastrointestinal tract. The release rate from CDS pellets in artificial gastric juice (1st fluid, Fig. 3B) was more rapid than that in the 2nd fluid. To ascertain the effect of the dispersed chitosan powder in the release-regulating layer, SR pellets in which the release-regulating layer was composed of Eudragit RS alone were prepared. Drug release profiles of SR pellets were not affected by the pH difference of the dissolution medium (Fig. 3). These results suggest that chitosan powder dispersed in the release-regulating layer dissolves in acidic conditions and the permeability of the drug to the layer increases in the 1st fluid. It is concluded that the dispersed chitosan powder in the release-regulating layer plays an important role in drug release from CDS pellets.

The gastrointestinal transit of pharmaceutical dosage forms was reported by Davis *et al.*[17] According to the literature, the average gastric emptying time and small intestinal transit time is 2 h and 3 h, respectively. Therefore release tests of CDS-7 h pellets were carried out in the 1st fluid for 2 h and immediately followed by the 2nd fluid for 3 h, *i.e.*, under simulated gastrointestinal conditions of pH and transit times as shown in Fig. 4. In spite of the drug release lag time from the preparations, the drug release rate increased for 1 h in the 1st fluid and the drug release rate was constant for 3 h in the 2nd fluid. After replacement of the medium with a pH 4.0 buffer solution, in which chitosan powder was easily dissolved, the release of drug markedly increased. It was recently reported that the pH in the colon is actually lower than that in the small intestine due to the acidification of colonic contents by products of bacterial fermentation.[18,19] The decrease in pH on entry into the colon is due to the presence of short-chain fatty acids arising from the bacterial fermentation of polysaccharides. Furthermore, the pH in the colon was reduced in a group of untreated ulcerative colitis disease patients and the pH in the colon often drops to between 1 and

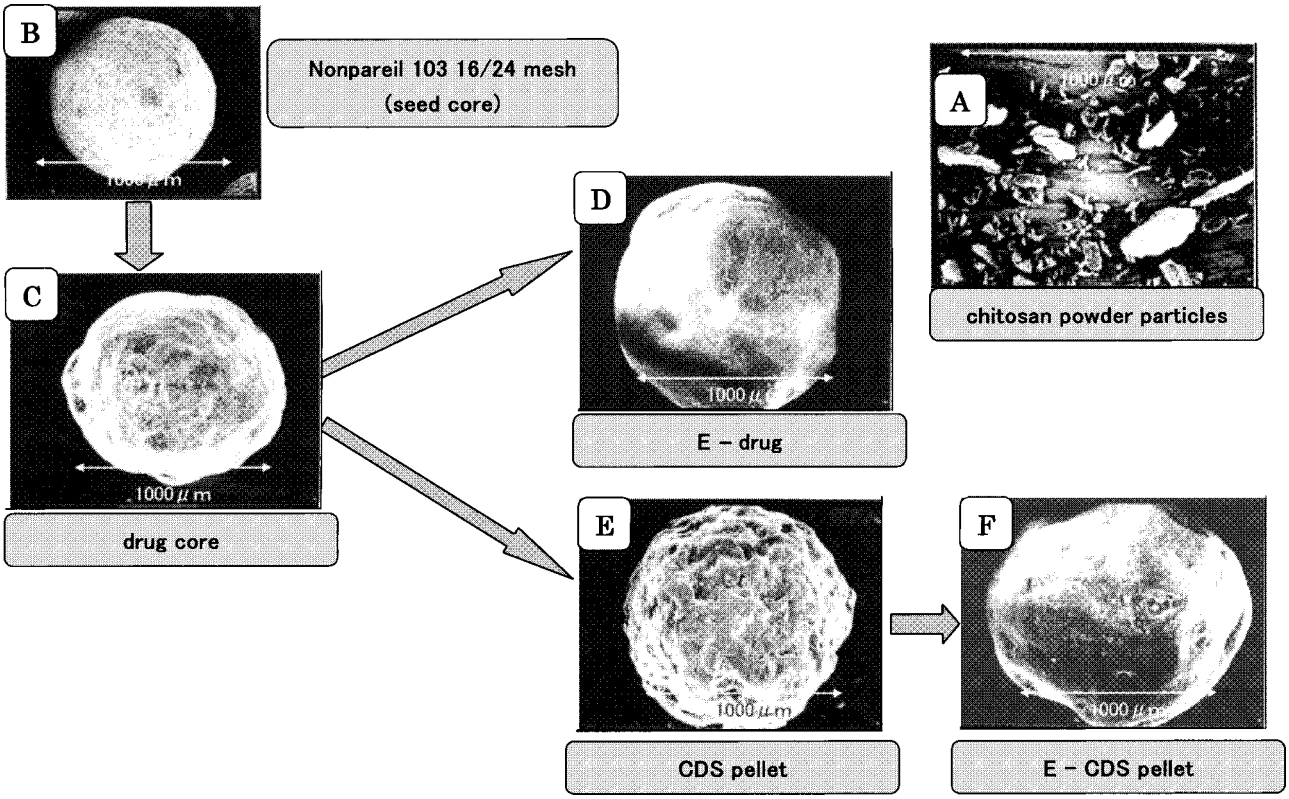

Fig. 2. Scanning Electron Microphotographs of (A) Chitosan Powder Particles, (B) Nonpareil (Seed Core), (C) Drug Core, (D) E-Drug, (E) CDS Pellets, and (F) E-CDS Pellets

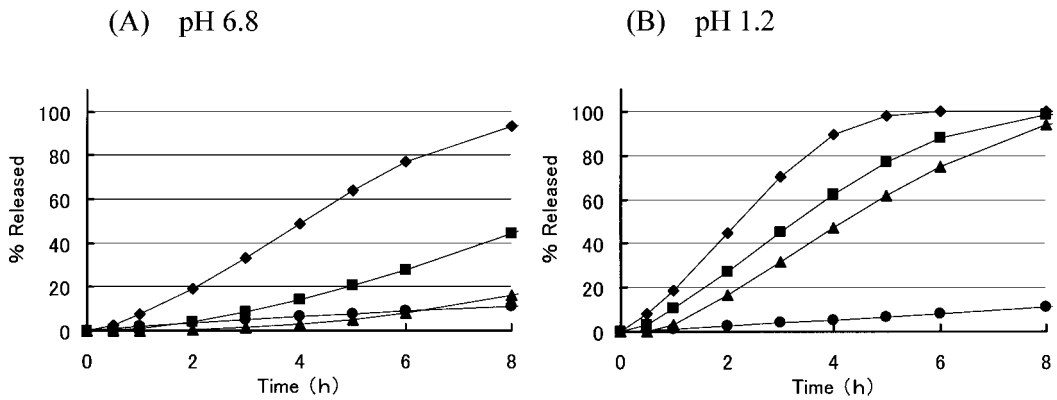

Fig. 3. Release Profiles of AAP from Various CDS Pellets

(A) 2nd fluid, 500 ml, rotating basket method, 100 rpm. (B) 1st fluid, 500 ml, rotating basket method, 100 rpm. Each point represents the mean value of 3 experiments. ◆, CDS-2 h; ■, CDS-4 h; ▲, CDS-7 h; ●, SR-pellets.

5.[19] It is considered that dissolution testing using pH 4.0 buffer reflects the release profile of AAP from CDS pellets when dispersed chitosan powder was degraded. When using rat cecal contents as the dissolution medium, the release profile of AAP from CDS pellets was affected by the conditions of cecal content. Therefore, from the viewpoint of simplification of the evaluation, pH 4.0 buffer solution in which chitosan powder was the most easily dissolved was used in this study.

(2) Morphological Examinations of CDS Pellets Treated with Rat Cecal Contents: Chitosan is known to be degraded by enzymes[14] and to be dissolved in acidic medium. Therefore to ascertain its effect, fluid in which rat cecal contents was suspended in the 2nd fluid (chitosan is not dissolved in this fluid) was prepared. One was heat-treated at 80 °C for 30 min and the other was not treated, because in general, enzymes are very sensitive to heat. The surfaces of each CDS pellet are shown in Fig. 5. Figure 5A shows the surface of intact CDS pellets, and Fig. 5B and 5C show the surface of CDS pellets which were treated with rat cecal contents. It was found that the surface of CDS pellets (A) was degraded when the rat cecal contents were not heat-treated (C). On the other hand, the surface of the CDS pellets did not change in the suspension which was heat-treated for 30 min at 80 °C (B). This means that chitosan powder in the release-regulating layer is degraded by contents that are sensitive to heat. It is therefore suggested that drug release from CDS pellets may increase when they are exposed to enzymes in the colon.

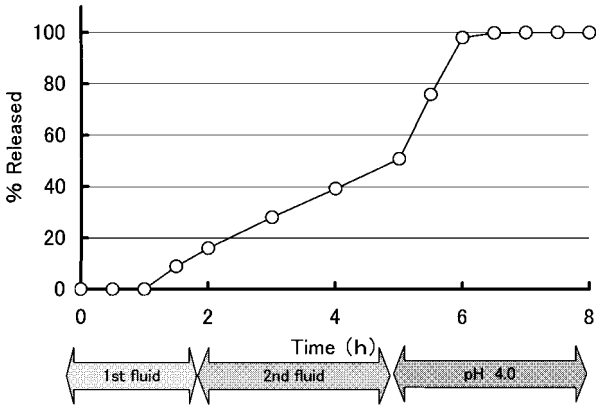

Fig. 4. Release Profile of AAP from CDS-7 h Pellets under Simulated Gastrointestinal Transit

Buffer, 500 ml, rotating basket method, 100 rpm. Each point represents the mean value of 3 experiments.

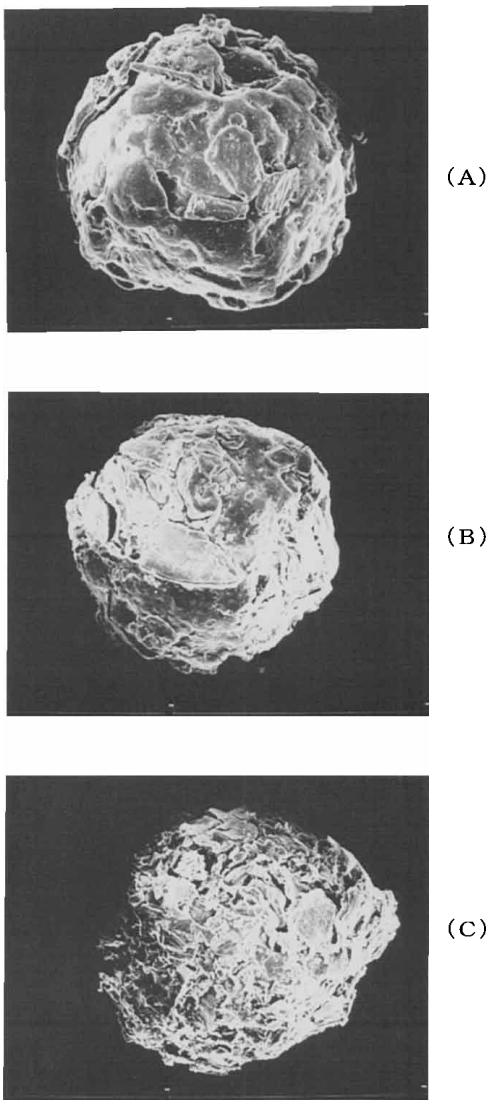

Fig. 5. Scanning Electron Microphotograph of the Surface of CDS Pellets

(A) Intact CDS pellets. (B) After release testing in the heat-treated suspension of cecal contents. (C) After release testing in the nonheat-treated suspension of cecal contents.

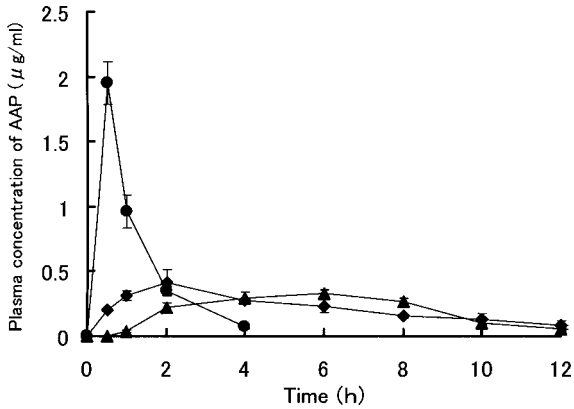

Fig. 6. Plasma Concentration *vs.* Time Curves of AAP (10 mg/kg) after Oral Administration of CDS Pellets in Rats

Results are expressed as the mean±S.E. of 3—6 rats. ●, solution (n=3); ◆, CDS-2 h (n=3); ▲, CDS-7 h (n=6).

Table 1. Pharmacokinetic Parameters of AAP in Rats after Oral Administration of Various Preparations at a Dose of 10 mg/kg

Preparation	T_{max} (h)	C_{max} (μg/ml)	*AUC* (μg·h/ml)	*MRT* (h)
Solution	0.3±0.0	3.14±0.22	3.07±0.16	1.4±0.1
CDS-2 h	2.7±0.6	0.43±0.08	3.13±0.17	5.9±0.7
CDS-7 h	4.7±0.8	0.38±0.03	2.72±0.15	6.6±0.3

Each value represents the mean±S.E. [Solution (n=3), CDS-2 h (n=3), CDS-7 h (CDS) (n=6)].

(3) *In Vivo* Evaluation of CDS Pellets: Figure 6 shows the plasma concentration *vs.* time curve of AAP after oral administration of AAP solution (solution), CDS-2 h, and CDS-7 h pellets to rats. Pharmacokinetic parameters are presented in Table 1. After oral administration of CDS pellets, decreased C_{max} and prolonged T_{max} and *MRT* were observed as compared with those after oral administration of solution. However, there were no statistically significant differences in the *AUC* between the three preparations. T_{max} and *MRT* increased with the increasing coating level of the release-regulating layer of CDS pellets.

Based on results of *in vitro* tests (Fig. 3), dispersed chitosan in CDS-2 h pellets was considered to dissolve in fair amounts in the stomach, and the drug might be released in the stomach and the small intestine. In the case of CDS-7 h pellets, it is considered that dispersed chitosan dissolves in part in the stomach, and the remaining chitosan is degraded in the colon, resulting in drug release in wide regions of the gastrointestinal tract, *i.e.*, from the stomach to the colon.

Colon-Specific Drug Delivery Using E-CDS Pellets CDS-7 h pellets released small amounts of drug for 2 h in the 1st fluid, but not for more than 3 h in the 2nd fluid (Fig. 3). For colon-specific drug delivery, E-CDS pellets were prepared by coating enteric polymer on CDS pellets and *in vitro* and *in vivo* evaluations were carried out.

(1) Dissolution Profile of E-CDS Pellets: Figure 7 shows release profiles of AAP from E-CDS pellets and E-Drug pellets under simulated gastrointestinal conditions of pH and transit times. E-Drug pellets were prepared by coating enteric polymer on drug cores without chitosan-dispersed layer. No drug release from E-CDS pellets was observed in the 1st

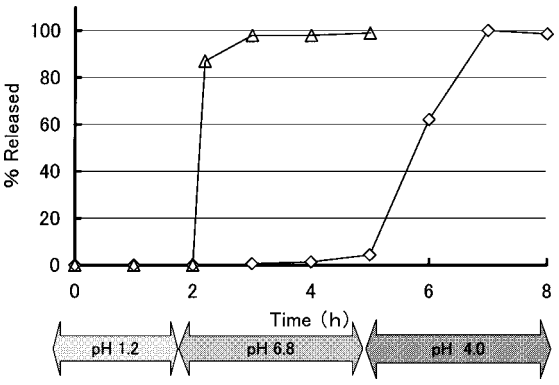

Fig. 7. Release Profiles of AAP from E-Drug and E-CDS Pellets

Buffer, 500 ml, rotating basket method, 100 rpm. Each point represents the mean value of 3—6 experiments. △, E-Drug ($n=3$); ◇, E-CDS pellets ($n=6$).

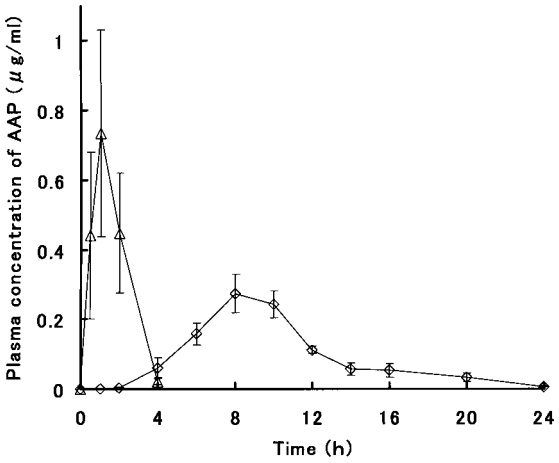

Fig. 8. Plasma Concentration *vs.* Time Curves of AAP (10 mg/kg) after Oral Administration of E-Drug and E-CDS Pellets in Rats

Results are expressed as the mean±S.E. of 5—6 rats. △, E-Drug ($n=5$); ◇, E-CDS pellets ($n=6$).

fluid more than 2 h, and after replacement of the medium with the 2nd fluid, almost no drug was released in the subsequent 3 h. Thereafter the drug was markedly released from E-CDS pellets when the medium was replaced with a pH 4.0 buffer, in which chitosan powder in the release-regulating layer was easily dissolved. E-Drug pellets released the drug immediately after replacement of medium with the 2nd fluid.

(2) *In Vivo* Evaluation of E-CDS Pellets: Figure 8 shows plasma concentration *vs.* time curves of AAP after oral administration of E-CDS and E-Drug pellets in rats, and pharmacokinetic parameters are listed in Table 2. Plasma levels of AAP were not detected within 4 h after oral administration of solution (Fig. 6) and E-Drug pellets. However, in the case of E-CDS pellets, AAP was detected in plasma for 4—24 h after oral administration. The T_{max} and *MRT* after E-CDS pellet dosing were 9.3 h and 10.0 h, respectively, which were significantly longer than those after administration of solution, CDS pellets (Table 1) and E-Drug pellets. This result was in good agreement with the *in vitro* dissolution characteristics of E-CDS pellets (Fig. 7). Therefore it is concluded that E-CDS pellets pass intact through the stomach and the small intestine, and when pellets reach the colon, chitosan in the release-regulating layer is dissolved under colonic conditions of pH or is degraded by enzymes,[14] resulting in colon-specific drug delivery.

Table 2. Pharmacokinetic Parameters of AAP in Rats after Oral Administration of Various Preparations at a Dose of 10 mg/kg

Preparation	T_{max} (h)	C_{max} (μg/ml)	*AUC* (μg·h/ml)	*MRT* (h)
E-Drug	1.1±0.2	0.89±0.25	1.59±0.51	1.9±0.2
E-CDS	9.3±0.7	0.33±0.05	2.11±0.07	10.0±0.7

Each value represents the mean±S.E. [E-Drug ($n=5$), E-CDS ($n=6$)].

Conclusions

In the present study, CDS was adopted for a multiparticulate system (CDS pellets). The drug release rate increased as chitosan powder dissolved in the stomach, but the drug release rate in the small intestine was constant and markedly increased in the colon. The drug release rate was controlled by the thickness of the chitosan-dispersed water-insoluble polymer. CDS pellets were found to be effective for long-term drug release in wide regions of the gastrointestinal tract. For colon-specific drug delivery, CDS pellets were further coated with an enteric polymer. The resultant enteric-coated CDS (E-CDS) pellets were found to be a useful drug delivery system for the colon.

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