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A Dissolution Test for a Pressure-controlled Colon Delivery Capsule: Rotating Beads Method

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Abstract

The rotating beads method is a new in-vitro dissolution test proposed for drugs formulated as pressure-controlled colon delivery capsules (PCDCs).

The apparatus consisted of a glass vessel (500 mL) containing 4-mm (i.d.) glass beads (5000, 10 000 or 15 000) and dissolution medium (0·067 M phosphate buffer, pH 7; 25, 50 or 100 mL) containing polyvinyl alcohol (PVA; 5, 10 or 20 w/v%) to simulate the viscosity of the colon. The vessel was rotated at 5, 10 or 25 rev min^{-1} and the temperature was maintained at 37°C. Fluorescein was used as a model drug to explore the optimized conditions under which differences in the drug dissolution rate are detected between colon delivery systems. Fluorescein was formulated in four types of colon delivery systems. One was a tablet coated with an enteric polymer, Eudragit S-100, and the other three were PCDCs prepared with different thicknesses of ethylcellulose coating membrane (type I, II, III).

The dissolution behaviour of fluorescein from the PCDC formulation was significantly different from that of the Eudragit S-100-coated tablets, when the dissolution conditions were as follows: rotation speed, 10 rev min^{-1}; bead number, 10 000; dissolution medium, 50 mL with 10% PVA. This dissolution method was applied to acetaminophen sustained-release tablets and two other drugs having low solubility in the colon, tegafur and 5-aminosalicylic acid. Similarly, significant differences in the dissolution rates of drugs from the PCDC formulation and the enteric tablet were detected. There was good correlation between the in-vitro dissolution rates and in-vivo absorption rates using T50 (the time for half of the amount of drug to be released from the preparation) and C_{max}/T_{max} (enteric tablet) or $C_{max}/(T_{max}-T_i)$ (PCDC), where T_i is the first appearance time in the systemic circulation.

The rotating beads method is a valuable technique for evaluating the dissolution rate of drugs formulated in PCDC.

Colon delivery systems are useful for the oral administration of peptide/protein drugs (Rubinstein et al 1997) as well as for drugs used in treating colon-specific diseases such as ulcerative colitis and Crohn's disease (Ashford & Fell 1994; Sayani & Chein 1996). Colon delivery systems are divided mainly into three categories, pH-sensitive drug dissolution (Ashford et al 1993), time-controlled drug dissolution (Industrial Pharmacists Group 1991; Gazzaniga et al 1994, 1995), and microbially-controlled drug dissolution (Saffran et al 1986; Wakerly et al 1997). However, we have been studying colon delivery systems which are based on a unique mechanism, intestinal luminal pressure-controlled colon delivery capsules (PCDCs) (Takaya et al 1995, 1997). The PCDC formulation is prepared by coating the inner surface of gelatin capsules with ethylcellulose. The thickness of the ethylcellulose coat determines the disintegration characteristics of the capsule, because the gelatin layer dissolves immediately after oral administration. In this colon delivery system, capsules are filled with drug molecules dissolved in a suppository base such as polyethylene glycol (PEG), Pharmasol or Witepsol. Therefore, once the gelatin dissolves, the system behaves like an ethylcellulose

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balloon containing drug solution because the suppository base dissolves at body temperature. In the upper part of the gastrointestinal tract, there is enough fluidity so that the ethylcellulose balloon is not directly subjected to intestinal luminal pressure (Digenis & Sandefer 1991; Ritschel 1991; Möse 1993). However, reabsorption of water occurs in the colon and the viscosity of the luminal contents increases. As a result, intestinal pressure due to peristalsis directly affects the ethylcellulose balloon. Since the ethylcellulose balloon cannot tolerate these pressures, it disintegrates in the intestine. By adjusting the thickness of the ethylcellulose coating membrane, colon delivery of several drugs such as 5-aminosalicylic acid, carbamazepine and tegafur has been successful (Takaya et al 1997). From those studies, a good correlation between the systemic availability of drugs and the dissolution rate of drug molecules from the colon delivery system has been noted. We suggested that the dissolution process was the critical factor for the systemic availability of drugs after delivery to the colon (Takaya et al 1998). Therefore, an in-vitro dissolution method is needed to predict the systemic availability of drugs administered by a colon delivery system. However, no in-vitro dissolution method for colon delivery systems has been reported. This is because little is known about the physical factors in the gastrointestinal tract. These physical factors include hydrodynamic flow and mechanical destructive forces that will arise from digestive actions (grinding or crushing of gastrointestinal tract contents) and/or friction between drug products and the gastrointestinal wall, although the effects of pH on drug dissolution rate has been extensively investigated (Hardy et al 1989). It is difficult to study the physical factors in the gastrointestinal tract in order to establish in-vitro testing systems with optimal destructive forces and hydrodynamic flow (Zahirul & Khan 1996). The aim of this study was to propose a new dissolution test method to examine the drug dissolution rate from colon delivery systems, especially the PCDC formulation, in comparison with other conventional systems such as pH-sensitive colon delivery where drug molecules are formulated into a solid mass, the enteric tablet, coated with Eudragit S-100.

Materials and Methods

Materials

5-Aminosalicylic acid was obtained from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan). Tegafur was obtained from Fuji Kagaku Kogyo Co., Ltd (Toyama, Japan). Gelatin capsules (#00, 24-mm long, 0·91 mL volume) were obtained from Yoshida Co., Ltd (Himeji, Japan). Ethylcellulose (7G grade) was a gift from Nisshin Chemical Industrial Co., Ltd (Osaka, Japan). Eudragit S-100 (Röhm Pharm, Damstadt, Germany) was obtained through Higuchi Inc. (Tokyo, Japan). Crystalline cellulose (Avicel, PH-101) was obtained from Asahi Kasei Kogyo Co., Ltd (Tokyo, Japan). Polyvinyl alcohol (POVAL C-05GP, PVA) was obtained from Shin-Etsu Chemical Co., Ltd (Tokyo, Japan). Polyethylene glycol (PEG) 1000, fluorescein and acetaminophen were obtained from Nacalai Tesque Inc. (Kyoto, Japan). Sorbitan monooleate (Tween 80) was obtained from Wako Pure Chemical Industry Ltd (Osaka, Japan).

Preparation of the PCDC formulation containing fluorescein, acetaminophen, tegafur and 5-aminosalicylic acid

The PCDC formulation was prepared as described previously (Takaya et al 1995, 1997). Briefly, 1·1 mg ethylcellulose was dissolved in 5 mL methylene chloride–methanol (4 : 1) mixture by stirring. Pores (2·0-mm diam.) were made at the top and the bottom of #00 gelatin capsules, and 180 μL ethylcellulose solution was added. The inner surfaces of the capsules were coated with the ethylcellulose solution by rotating horizontally at 30°C for 12 h. The thickness of the ethylcellulose coating membrane was $44{\cdot}7 \pm 0{\cdot}9$ μm (type II). For the pilot fluorescein study two more types of PCDC were prepared. Type I capsules had a thinner ethylcellulose membrane of $27{\cdot}3 \pm 0{\cdot}9$ μm and type III had a thicker membrane of $63{\cdot}3 \pm 0{\cdot}3$ μm. The pores at the bottom of the capsule bodies were sealed with concentrated ethylcellulose solution (ethylcellulose glue). A sample of drug (30 mg fluorescein, 150 mg acetaminophen, 10 mg tegafur or 250 mg 5-aminosalicylic acid) was dissolved with 0·9 mL PEG 1000 at 50°C, and the solution was poured through the pore at the top of the PCDC (type II). The remaining void space was filled with warm PEG 1000 solution. After the PEG 1000 had formed a hard mass, the pore at the top of the capsule was sealed with ethylcellulose glue.

Preparation of Eudragit S-100 coated tablets

Tablets containing fluorescein, acetaminophen, tegafur or 5-aminosalicylic acid were prepared. Each drug (30 mg fluorescein, 150 mg acetaminophen, 10 mg tegafur or 250 mg 5-aminosalicylic acid) was mixed with Avicel (PH-101) of

470, 350, 490 or 250 mg, respectively. Tablets (average weight 500 mg) containing drug and Avicel (PH-101) were compressed at an applied force of 150 N and a compression time of 5 s using 13-mm round, flat and plain punches on a single station tableting machine (Hand press, Model SSP-10A, Shimadzu, Japan). Enteric coating solution was prepared by dissolving 1 mg Eudragit S-100 with 10 mL methylene chloride–methanol (4 : 1) mixture. Tablets were coated using a dipping method. The thickness of the Eudragit S-100 coating membrane was approximately 70 μm.

In-vitro dissolution tests

The in-vitro dissolution characteristics of each drug from the PCDC formulation or Eudragit S-100 coated tablets were determined using the apparatus shown in Figure 1. The apparatus consisted of a 500-mL glass vessel (length 15·5 cm; i.d. 6·5 cm) containing 5000, 10 000 or 15 000 glass beads of 4-mm diameter. This vessel was fixed horizontally with a stainless bar as an axis and was rotated vertically to the axis during the dissolution test. The dissolution medium used was 25, 50 or 100 mL 0·067 M phosphate buffer (pH 7) with 5, 10 or 20 w/v% PVA. The dissolution medium for 5-aminosalicylic acid contained 0·2 w/v% Tween 80 because it is a water-insoluble drug. The vessel was rotated at 5, 10 or 25 rev min^{-1} and a temperature of 37°C was maintained throughout the study. Dissolution tests were performed for 6 h. For the PCDC formulations a 400-μL portion of each sample was taken at 10, 20, 30, 40, 50, 60, 80, 100, 120, 150, 180, 210, 240, 300, and 360 min. For the enteric tablet, samples (400 μL) were taken at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 240, 300, and 360 min. After centrifugation at 12 000 rev min^{-1} for 5 min to remove solid components ascribed to the PCDC formulation or the enteric tablet, 200 μL portions of each sample were transferred to clean capped glass tubes and were frozen at −20°C until assay.

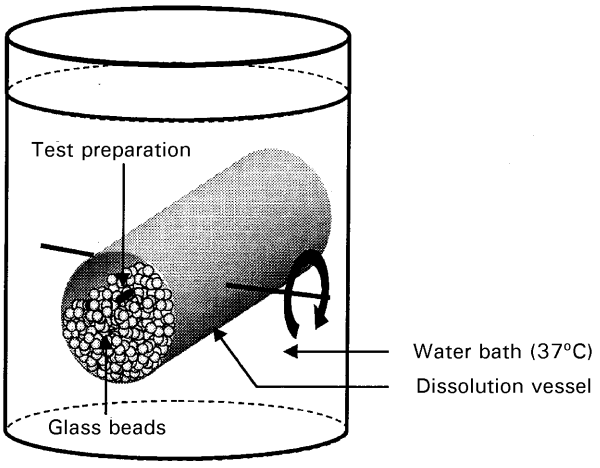

Figure 1. Dissolution apparatus of the rotating beads method for the colon delivery system.

Analytical procedures

Fluorescein. Two hundred microlitres of the dissolution test sample was diluted with 3 mL distilled water. The amount of dissolved fluorescein was determined spectrophotometrically at 490 nm using a Shimadzu spectrophotometer (UV-240, Shimadzu, Kyoto, Japan). Fluorescein concentrations were determined from calibration curves. The standard curve of fluorescein added to the dissolution medium was linear over the range of 0·1–0·6 mg mL^{-1} and passed through the origin.

Acetaminophen. Two hundred microlitres of the dissolution test sample was diluted with 1 mL distilled water. The acetaminophen concentration was determined by an HPLC method. The separation on an octadecylsilane column (TSKgel ODS-80TS, 4·6 mm × 150 mm, 5 μm; Tosoh, Tokyo, Japan) was achieved at 50°C at a flow rate of 0·8 mL min^{-1}. The mobile phase consisted of water/acetonitrile/methanol (88 : 6 : 6, v/v). UV detection was carried out at 254 nm. Fifty microlitres of each sample was injected into the HPLC system using a Shimadzu auto-sampler, Model SIL-6A. The system was composed of a Shimadzu LC 10-AS pump, Model SPD-10A UV detector, Shimadzu CTO-2A column oven and Chromatopac CR-4A data processor. The acetaminophen concentrations were determined from calibration curves. The standard curve of acetaminophen added to the dissolution medium was linear over the range of 0·1–5 mg mL^{-1} and passed through the origin.

Tegafur. Two hundred microlitres of the dissolution test samples of tegafur were diluted with 1 mL water and 50 μL of each sample was injected into the HPLC system using an auto-injector, Tosoh Model AS-8010 (Tokyo, Japan). The analytical column was TSKgel ODS-80TS (4·6 mm × 150 mm, Tosoh). The mobile phase was acetonitrile/water (10 : 90) and the pH was adjusted to 3·0 by the addition of trifluoroacetic acid. The flow rate was 0·8 mL min^{-1}. Analysis was performed at 65°C. The UV detection wavelength used was 270 nm. Tegafur concentrations were determined from calibration curves. The standard curve of

tegafur added to the dissolution medium was linear over the range of 0·1–1 mg mL^{-1} and passed through the origin.

5-Aminosalicylic acid. One hundred microlitres of the dissolution test samples of 5-aminosaliclic acid were diluted with 1 mL methanol and 30 μL of each sample was injected into the HPLC system. The HPLC analytical method was the same as previously reported (Takaya et al 1997). Briefly, a reversed-phase HPLC method using Chemcosorb 7C8 (4·6 mm i.d. × 250 mm length) column (Chemco Scientific, Osaka, Japan) was used. 5-Aminosalicylic acid eluted from the analytical column was detected by a fluorescence spectrophotomonitor at an excitation wavelength of 315 nm and an emission of 460 nm. 5-Aminosalicylic acid concentrations were determined from calibration curves. The standard curve of 5-aminosalicylic acid added to the dissolution medium was linear over the range of 0·1–5 mg mL^{-1} and passed through the origin.

Statistics

All values are expressed as the mean ± s.e. Statistical differences were assumed to be reproducible when $P < 0{\cdot}05$ (one-sided *t*-test).

Results and Discussion

Explorative experiment on dissolution test conditions

To optimize the dissolution test conditions, we studied the effects of several features of the experimental apparatus (Figure 1) on the dissolution characteristics of drugs from colon delivery systems; number of beads, rotation speed, amount of added PVA and volume of dissolution medium using the three types of PCDC formulation (type I, II, III), and the enteric tablets coated with Eudragit S-100. First, it was necessary to explore the conditions of the proposed dissolution test method by which the difference in drug dissolution rate could be obtained between the PCDC formulation and the enteric tablet. For this optimization study, fluorescein was used as a model of a poorly water-soluble drug. Since nutrients are absorbed in the small intestine and water is reabsorbed in the colon, the viscosity of the luminal contents of the colon is higher than that of the small intestine. The hydrodynamic flow in the colon luminal space is also decreased. Initially, the conditions of this proposed dissolution method were set at 10 000 glass beads, 50 mL 10% PVA in phosphate buffer as the dissolution medium, and a rotation speed 10 rev min^{-1}. By varying each factor we explored the appropriate condition for the dissolution study. Except for the varied factor, the others were set on the same level as the tentative conditions. The rotation speed of the glass vessel was varied from 5 to 25 rev min^{-1}. Figure 2A shows the results of the dissolution profiles of fluorescein from the PCDC formulation (type II) and the enteric tablet. The comparison of the dissolution profiles from the three types of PCDC formulation (type I, II, III) are shown in Figure 2B. The luminal pH of the colon is reported to be approximately 7·0 (Evans et al 1986; Lui et al 1986). Eudragit S-100, which is representative of enteric polymers and has a threshold pH of 6·8 (Röhm Pharm 1995), has been used as a pH-sensitive polymer for the colon targeting of drugs, although the specificity of colon targeting is vague. Azo-polymer (Saffran et al 1986), which receives the enzymatic degradation by azo-reductase from the microflora in the colon, is thought to have higher colon targeting specificity than Eudragit S-100. However, the faeces were not added to the dissolution medium in our proposed dissolution system. Therefore, a pH-sensitive colon delivery system was used as the conventional colon targeting tablet and not azo-polymer-coated tablets. With rotation speeds of 10 and 25 rev min^{-1}, fluorescein was dissolved instantaneously from the PCDC formulation but the dissolution rate from the enteric tablet was slower. When the rotation speed was decreased to 5 rev min^{-1}, the dissolution rate of fluorescein from the PCDC formulation was prolonged. As the PCDC formulation was formulated with PEG 1000 as a suppository base, fluorescein was dissolved in the capsule at body temperature after oral administration. Once the PCDC formulation disintegrates in the colon as a result of the colon luminal pressure caus PCDC formulation instantaneously. Therefore, 5 rev min^{-1} seemed to be inappropriate for the simulation of the drug dissolution process in the colon. Of the three rotation speeds the difference of the values of T50 (the time for half of the amount of drug to be released from the preparation) between the two formulations was largest at 10 rev min^{-1} and so this speed was selected as the most suitable to reflect the difference in the dissolution rates of fluorescein from the PCDC formulation and the enteric coated tablet. There was no significant difference in the dissolution profiles from type I and type II capsules and that of type III represented slower release. However, rotation speed seemed to affect the dissolution profile dependent on the thickness of the ethylcellulose coating membrane.

The effect of the number of glass beads in the dissolution vessel on the dissolution rate of fluorescein was studied and the results are shown in Figure 3. In Figure 3A, when the number of glass beads was 10 000, the dissolution of fluorescein from the PCDC formulation (type II) occurred instantaneously. However, when the number of glass beads was increased to 15 000 or decreased to 5000, the dissolution rate of fluorescein from the PCDC formulation was prolonged. When the number of beads was 5000, it was thought that the force on the PCDC formulation was less than that with a glass bead number of 10 000. When the number of glass beads was increased to 15 000, insufficient water and the increase of the mechan-

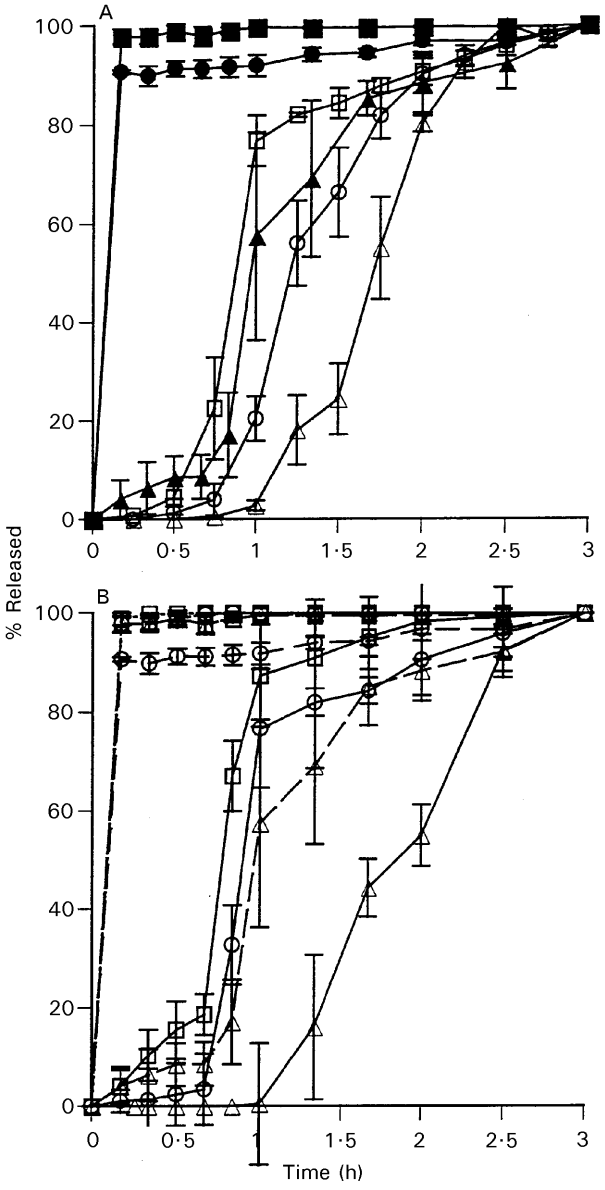

Figure 2. A. Effect of rotation speed on the dissolution profile of fluorescein from the PCDC formulation (closed symbols) and the enteric tablet (open symbols). Each point represents the mean ± s.e. (n = 3). △, ▲, 5 rev min^{-1}; ●, ○, 10 rev min^{-1}; ■, □, 25 rev min^{-1}. B. Effect of rotation speed on the dissolution profile of fluorescein from PCDC formulations of various thickness (·····, type I; - - -, type II; –, type III). Each point represents the mean ± s.e. (n = 3). △, 5 rev min^{-1}; ○, 10 rev min^{-1}; □, 25 rev min^{-1}. Dissolution test conditions were; 10 000 glass beads, and 50 mL 10% PVA dissolution medium in phosphate buffer.

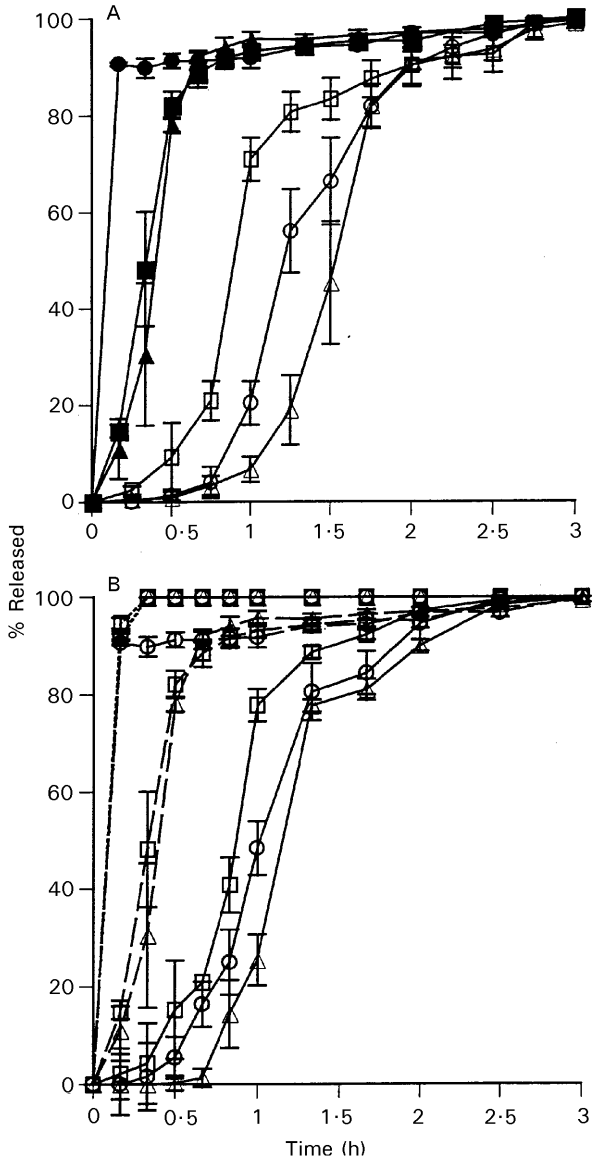

Figure 3. A. Effect of bead number on the dissolution profile of fluorescein from the PCDC formulation (closed symbols) and the enteric tablet (open symbols). Each point represents the mean ± s.e. (n = 3). △, ▲, 5000; ●, ○, 10 000; ■, □, 15 000. B. Effect of bead number on the dissolution profile of fluorescein from the various thicknesses of PCDC formulation (·····, type I; - - -, type II; –, type III). Each point represents the mean ± s.e. (n = 3). △, 5000; ○, 10 000; □, 15 000. Dissolution test conditions were; 10 rev min^{-1}, and 50 mL 10% PVA dissolution medium in phosphate buffer.

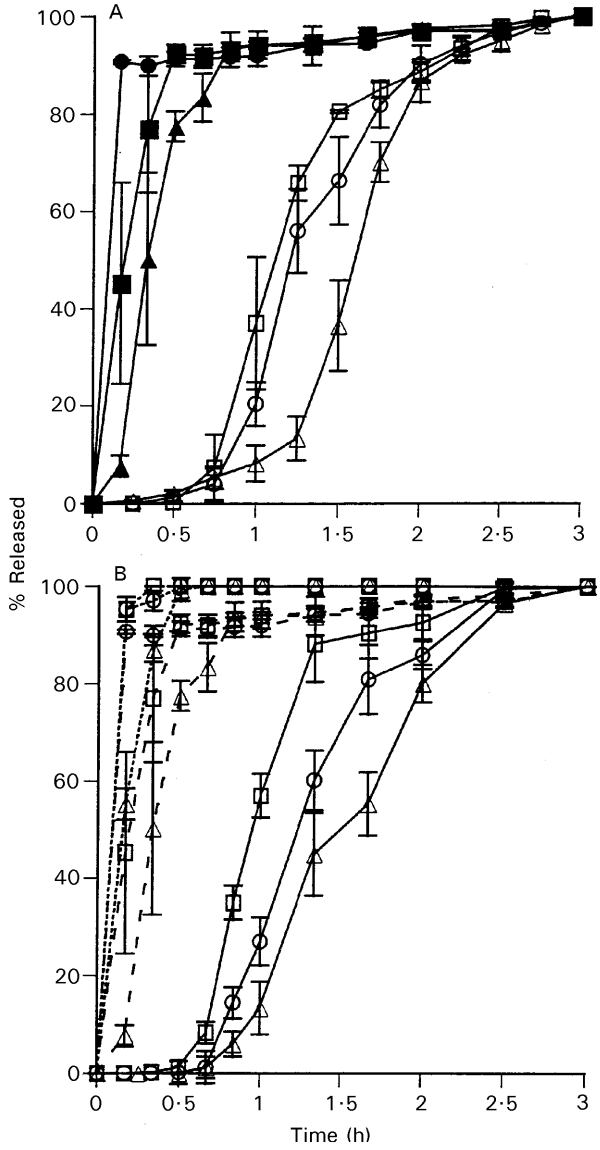

Figure 4. A. Effect of dissolution medium volume on the dissolution profile of fluorescein from the PCDC formulation (closed symbols) and the enteric tablet (open symbols). Each point represents the mean ± s.e. (n = 3). △, ▲, 25 mL; ●, ○, 50 mL; ■, □, 100 mL. B. Effect of dissolution medium volume on the dissolution profile of fluorescein from the various thicknesses of PCDC formulation (·····, type I; - - -, type II; –, type III). Each point represents the mean ± s.e. (n = 3). △, 25 mL; ○, 50 mL; □, 100 mL. Dissolution test conditions were; 10 rev min^{-1}, 10 000 glass beads, and 10% PVA dissolution medium in phosphate buffer.

ical force caused slow release profiles for both preparations. Therefore, the dissolution rate of fluorescein from the PCDC formulation was thought to be prolonged Thus we determined that a glass bead number of 10 000 was appropriate for the evaluation of drug dissolution rates from colon delivery systems. As shown in Figure 3B, as well as bead number, the dissolution profiles from the three types of PCDC formulation showed dependency on the thickness of the ethylcellulose coating membrane. However, considering the reproducibility of the dissolution test, 10 000 glass beads was thought appropriate.

The effect of dissolution medium volume on the dissolution rate of fluorescein was investigated and the results are shown in Figure 4. Unlike rotation speed and bead number, there were no significant differences between the dissolution profiles of the PCDC formulation (type II) from the three medium volumes (Figure 4A). Although not significant, when 25 mL dissolution medium was used, the dissolution profile of the PCDC formulation was slower than in the two other volumes of medium. Since drug dissolution should occur immediately in the body, this volume seemed not to reflect the dissolution rate of the PCDC formulation. Similarly, the enteric tablet in 25 mL dissolution medium resulted in the a lowest dissolution rate. This may be due to a lower chance of contact between the dissolution medium and the glass beads. In the case of 100 mL dissolution medium, the dissolution rate of the PCDC formulation was moderate, whereas that of the enteric tablet was too fast and the reproducibility was lower than with the other two volumes. The suitable condition needs the larger difference between the two formulations, and the appropriate reproducibility. Therefore, 50 mL dissolution medium was thought to be the most suit-able for the dissolution studies. Similarly, as shown in Figure 4B, the dissolution rates of the three types of PCDC formulation were shown to be in the order $25 < 50 < 100$ mL over the three thicknesses of ethylcellulose membrane. However, in the case of 25 and 100 mL, good reproducibility could not be obtained. Thus, we selected 50 mL as the appropriate volume for the dissolution medium.

The effect of PVA concentration in the dissolution medium on the dissolution rate of fluorescein from the PCDC formulation (type II) and the enteric tablet was studied and the results are shown in Figure 5A. As the formation of faeces starts in the colon, the motility pattern of the colon differs from that of other parts of the intestine, especially the small intestine. To represent the motility of the colon, glass beads and a highly viscous dissolution medium containing PVA were used in this study. PVA is a water-soluble polymer and was used to increase the viscosity of the dissolution medium at a concentration ranging from 5 to 20 w/v%. The viscosity of the three different PVA concentrations were 4×10 mPa·s (5 w/v%), 4×10^2 mPa·s (10 w/v%), and 3×10^3 mPa·s (20 w/v%) (Shin-Etsu Chemical 1998). When the PVA concentration was 5 and 10 w/v%, fluorescein was released immedi-

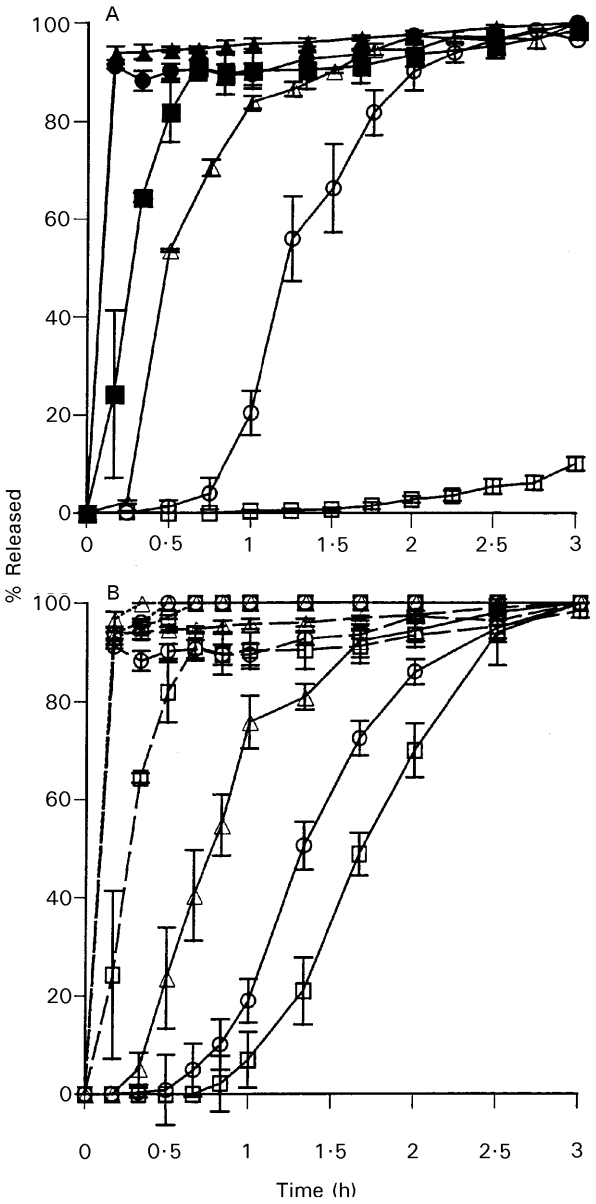

Figure 5. A. Effect of PVA concentration in the dissolution medium on the dissolution profile of fluorescein from the PCDC formulation (closed symbols) and the enteric tablet (open symbols). Each point represents the mean ± s.e. (n = 3). △, ▲, 5%; ●, ○, 10%; ■, □, 20%. B. Effect of PVA concentration in the dissolution medium on the dissolution profile of fluorescein from the various thicknesses of PCDC formulation (·····, type I; - - -, type II; –, type III). Each point represents the mean ± s.e. (n = 3). △, 5%; ○, 10%; □, 20%. Dissolution test conditions were; 10 rev min^{-1}, 10 000 glass beads, and 50 mL PVA dissolution medium in phosphate buffer.

ately from the PCDC formulation. However, increasing the PVA concentration to 20 w/v%, slightly decreased the dissolution rate of fluorescein from the PCDC formulation, and the dissolution of fluorescein from the enteric tablet did not occur for at least 1 h after the start of the dissolution study. Using 5 w/v% PVA concentration, the dissolution rate of the enteric tablet was too fast to simulate the situation in the body. Therefore, 10 w/v% PVA concentration was selected as the suitable level, because the dissolution rate of the PCDC formulation was immediate and that of the enteric tablet was appropriate. As shown in Figure 5B, 20 w/v% PVA concentration resulted in the slowest release profiles observed. All the dissolution profiles from three types of PCDC formulation were dependent on the thickness of the ethylcellulose coating membrane in the three PVA concentrations. However, the reproducibility was not acceptable for the 5 or 20 w/v% PVA concentrations. Therefore, 10 w/v% PVA concentration was chosen as appropriate.

Thus, in our in-vitro dissolution experiment the most appropriate conditions to simulate the in-vivo dissolution behaviour of fluorescein from the PCDC formulation just after delivery to the colon were as follows: rotation speed, 10 rev min^{-1}; number of glass beads, 10 000; dissolution medium volume, 50 mL; PVA concentration in the dissolution medium, 10 w/v%.

Application of the rotating beads method to acetaminophen

Acetaminophen is a representative analgesic and several sustained-release acetaminophen preparations have been used clinically. However, it was reported by deconvolution analysis that the absorption of acetaminophen ceases at approximately 3 h after oral administration to beagle dogs.

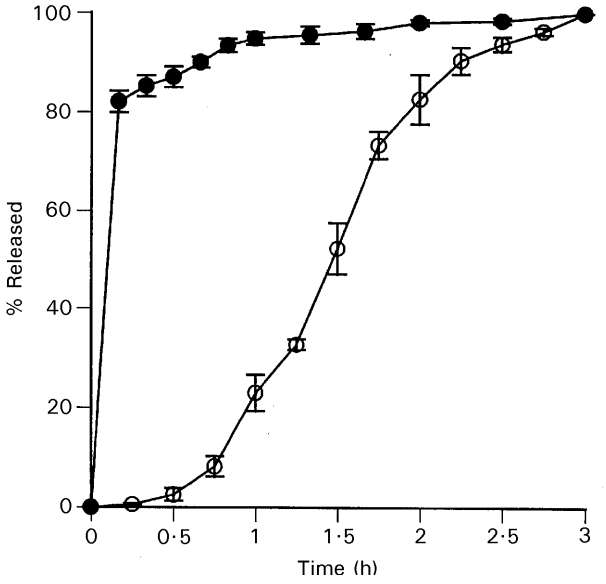

Figure 6. Comparison of the dissolution pattern of acetaminophen from the PCDC formulation (●) and the enteric tablet (○). Each point represents the mean ± s.e. (n = 3). Dissolution test conditions were; 5 rev min^{-1}, 10 000 glass beads, and 50 mL 10% PVA dissolution medium in phosphate buffer.

Sako et al (1996) speculated that the dissolution rate of acetaminophen was decreased just after delivery to the colon. Moreover, to simulate the dissolution profile in the later phase of absorption, a new dissolution method should be established, which has a low volume dissolution medium or a limited surface of the preparation. Taking this into consideration, we applied the rotating beads method to acetaminophen. The results are shown in Figure 6. Similar to fluorescein, the dissolution rates of acetaminophen from the PCDC formulation and the enteric tablet significantly differed. Although Sako et al (1996) stated that it was impossible to reflect the slow release of acetaminophen from controlled-release tablets in the colon using the JPXII paddle method, the difference in the dissolution rates of acetaminophen from the PCDC formulation and the enteric tablet were detected by the rotating beads method.

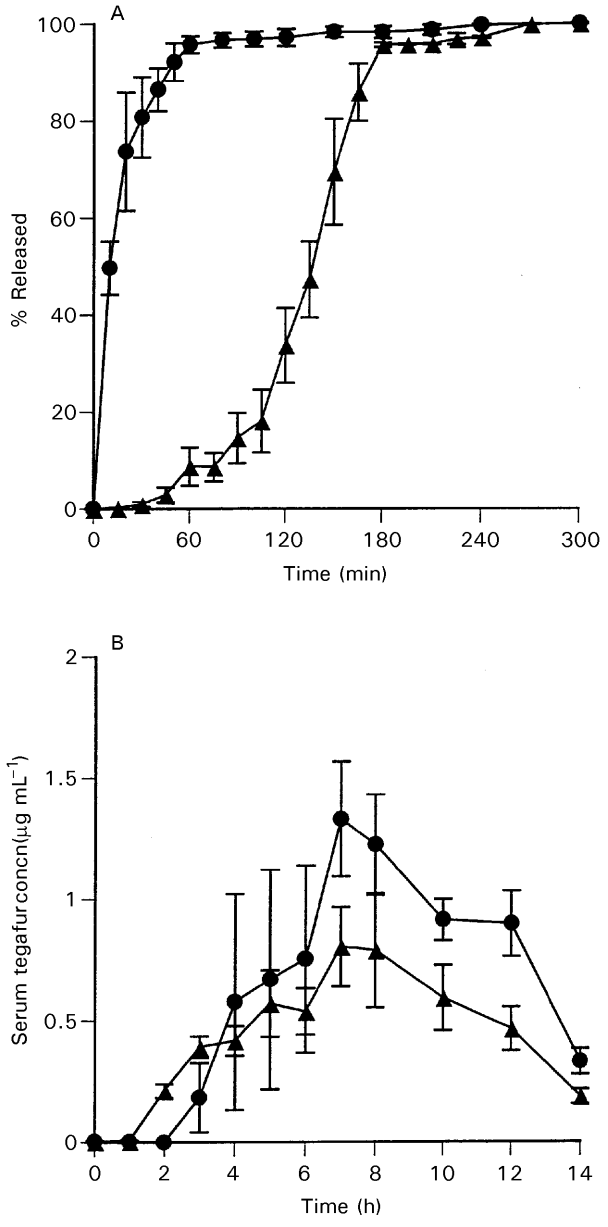

Figure 7. Relationship between in-vitro dissolution experiment and in-vivo pharmacokinetic study using two kinds of tegafur preparations, the PCDC formulation (●) and the enteric tablet (▲). A. In-vitro dissolution profiles by a rotating beads method. Each point represents the mean ± s.e. (n = 3). B. Mean serum tegafur concentration vs time profiles after oral administration to three beagle dogs, 10 mg/head.

Comparison of in-vitro dissolution test and in-vivo absorption study

No dissolution method has been reported that creates an environment which is closely related to the actual in-vivo conditions, particularly for colon delivery systems which show different dissolution/absorption profiles as compared with conventional dosage forms. We applied the proposed dissolution method to tegafur and 5-aminosalicylic acid, both of which have bioavailability problems because of low solubility after being delivered to the colon (Takaya et al 1998). The relationship between the in-vitro dissolution experiment and the in-vivo drug dissolution/absorption rate in/from the gastrointestinal tract was also studied. Figure 7 shows both in-vitro and in-vivo results with tegafur. Figure 7B shows the mean serum tegafur concentration–time curves in three beagle dogs administered tegafur in the enteric tablet and the PCDC formulation in our previous report (Takaya et al 1998). After the administration of the PCDC formulation, tegafur appeared in the systemic circulation with an absorption lag-time of approximately 3 h, which corresponds to the colon arrival time in these dogs as determined by sulfasalazine tests. Since the plasma tegafur concentration increased rapidly after oral administration of tegafur in the PCDC formulation, the dissolution rate of tegafur from the PCDC formulation is thought to be rapid. After administration of the enteric tablet to beagle dogs, the plasma tegafur concentration increased gradually and reached its peak concentration at about 8 h. Tegafur appeared to be released slowly from the enteric tablet in the lower part of the gastrointestinal tract. Figure 7A shows the results of the in-vitro dissolution experiment with the rotating beads method. The in-vitro dissolution experiment showed differences between the dissolution rates of the two preparations of tegafur. The dissolution rate of tegafur from the PCDC formulation was faster than that from the enteric tablet. The T50, the time for half of the amount of tegafur to be released from the preparation, was determined as the index of the in-vitro

dissolution rate, and for the enteric tablet the lag-time, which was determined as the sampling time just before the first appearance of drug into the dissolution medium, was excluded. The T50 of the PCDC formulation and the enteric tablet was 16·3 ± 6·6 and 86·6 ± 7·8 min, respectively.

The dissolution method was then applied to 5-aminosalicylic acid preparations. In-vivo results on the absorption kinetics of 5-aminosalicylic acid from the two preparations cited from Takaya et al (1998) and in-vitro release profiles are shown in Figure 8. In-vitro dissolution profiles of 5-aminosalicylic acid (Figure 8A) suggested that the drug was released faster from the PCDC formulation (T50 16·7 ± 1·7 min) than from the enteric tablet (T50 97·6 ± 3·2 min). Two hours after administration of enteric tablets mean plasma 5-aminosalicylic acid concentrations started to increase, and reached peak concentrations at approximately 3 h. After administration of the PCDC formulation, mean plasma 5-aminosalicylic acid concentration increased rapidly just after the absorption lag-time of 3 h, which is equal to the colon arrival time in beagle dogs, and reached its peak concentration immediately. These in-vivo absorption characteristics of 5-aminosalicylic acid into the systemic circulation seemed to reflect the in-vitro dissolution profiles of the two preparations.

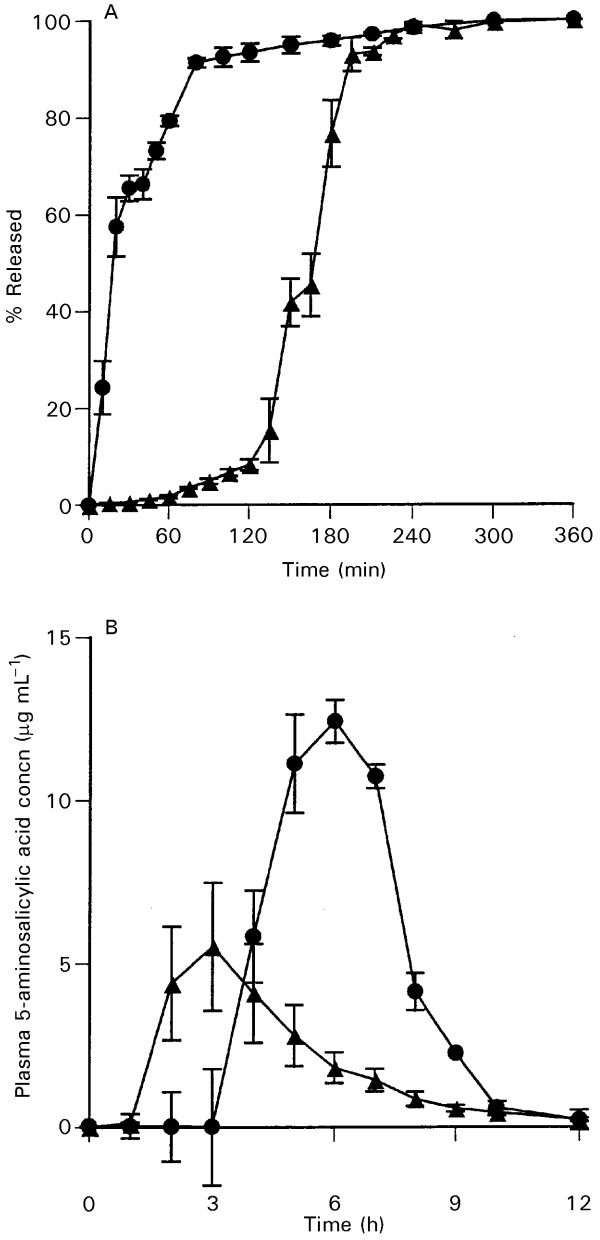

Figure 8. Relationship between in-vitro dissolution experiment and in-vivo pharmacokinetic study using two kinds of 5-aminosalicylic acid preparations, the PCDC formulation (●), and the enteric tablet (▲). A. In-vitro dissolution profiles by a rotating beads method. Each point represents the mean ± s.e. (n = 3). B. Mean plasma 5-aminosalicylic acid concentration vs time profiles after oral administration to three beagle dogs, 250 mg/head.

To characterize the relationship between the in-vitro dissolution experiment and in-vivo dissolution/absorption study, the relationship between T50 in the in-vitro dissolution experiment and the in-vivo dissolution/absorption rate was compared for tegafur and 5-aminosalicylic acid (Table 1). To estimate the in-vivo dissolution/absorption rate, the first-order absorption rate constant, k_a, determined by a compartmental pharmacokinetic analysis was considered to be a good parameter (Gibaldi & Perrier 1982). However, as the dissolution/absorption rate of each drug was very rapid from the PCDC formulation, it was impossible to take several blood samples during the absorption phase. Therefore, compartmental analysis could not be applied to the obtained data. As an in-vivo dissolution/absorption rate, C_{max}/T_{max} or $C_{max}/(T_{max}-T_i)$ was calculated for the enteric tablet or the PCDC formulation, where T_i is the first appearance time of drug in the systemic circulation,

Table 1. Comparison of the in-vitro and the in-vivo data from dissolution studies for tegafur and 5-aminosalicylic acid.

Drugs	Preparations	T50 (min)	C_{max}/T_{max} ($\mu g\,mL^{-1}\,h^{-1}$)
Tegafur	PCDC	16·3 ± 6·57	0·8 ± 0·49
	Enteric tablets	86·6 ± 7·77	0·1 ± 0·01
5-Aminosalicylic acid	PCDC	16·7 ± 1·67	12·5 ± 3·21
	Enteric tablets	97·6 ± 3·17	1·8 ± 0·65

T50 represents the index for the in-vitro experiment, which means the time required for 50% release. In the case of the enteric tablet, lag time was excluded. C_{max}/T_{max} represents the index for the in-vivo experiment. In the case of PCDC, it means $C_{max}/(T_{max}-T_i)$, where T_i means the lag time. Each value represents the mean ± s.e.

because in the PCDC formulation, the absorption lag-time was observed due to colon delivery. For tegafur the mean C_{max} and $(T_{max}-T_i)$ or T_{max} were $1{\cdot}5 \pm 0{\cdot}2\ \mu g\ mL^{-1}$ and $3{\cdot}3 \pm 1{\cdot}5$ h, respectively, for the PCDC formulation, and $0{\cdot}9 \pm 0{\cdot}2\ \mu g\ mL^{-1}$ and $7{\cdot}0 \pm 0{\cdot}6$ h, respectively, for the enteric tablet. For 5-aminosalicylic acid the mean C_{max} and $(T_{max}-T_i)$ or T_{max} were $16{\cdot}5 \pm 1{\cdot}7\ \mu g\ mL^{-1}$ and $1{\cdot}7 \pm 0{\cdot}7$ h, respectively, for the PCDC formulation, and $5{\cdot}5 \pm 2{\cdot}0\ \mu g\ mL^{-1}$ and $3{\cdot}0 \pm 0{\cdot}0$ h, respectively, for the enteric tablet. The mean $C_{max}/(T_{max}-T_i)$ and C_{max}/T_{max} of tegafur were $0{\cdot}8 \pm 0{\cdot}49\ \mu g\ mL^{-1}\ h^{-1}$ for the PCDC formulation and $0{\cdot}1 \pm 0{\cdot}01\ \mu g\ mL^{-1}\ h^{-1}$ for the enteric tablet, respectively. For 5-aminosalicylic acid the mean $C_{max}/(T_{max}-T_i)$ and C_{max}/T_{max} were $12{\cdot}5 \pm 3{\cdot}21\ \mu g\ mL^{-1}\ h^{-1}$ for the PCDC formulation and $1{\cdot}8 \pm 0{\cdot}65\ \mu g\ mL^{-1}\ h^{-1}$ for the enteric tablet, respectively. For tegafur, the in-vitro mean T50 values were 16·3 and 86·6 min for the PCDC formulation and the enteric tablet, respectively. For 5-aminosalicylic acid the values were 16·7 and 97·6 min for the PCDC formulation and the enteric tablet, respectively, although the value of the enteric tablet excludes the lag-time. As a result, there was a correlation between the in-vitro dissolution rate and the in-vivo dissolution/absorption rate.

Apparatus for extended dissolution preparations have been developed such as the closed systems of the paddle or basket methods. However, these systems are not suitable for dosage forms with very low solubility drugs because of the amount of water used and their limited ability to maintain sink conditions for the drug (Zahirul & Khan 1996). Komuro et al (1991) developed another dissolution technique, an open system flow-through cell method. This flow-through method had a demonstrable superiority over the paddle or basket methods for testing dissolution profiles of extended dosage forms. In addition, as a combination of basket and flow-through cell techniques, a rotating dialysis cell method was designed and was found to be useful in simulating food-induced factors during dissolution studies (El-Arini et al 1990). However, in the colon, less water is present than in the small intestine. Narisawa et al (1995) demonstrated that drug solubility strongly affected the dissolution rate in the lower site of canine gastrointestinal tract. Using a gamma scintigraphic study, Olsson et al (1995) suggested that the release of morphine from matrix tablets was too slow both in the distal small intestine and in the colon, and subsequently reduced bioavailability was obtained. Murata et al (1998) showed that solubility strongly affects the dissolution rate of diclofenac sodium in the lower site of the canine gastrointestinal tract. Therefore, to evaluate the in-vitro dissolution profiles for colon delivery systems, a different technique from the other methods described was required to overcome the problem of low quantities of water. Another modified apparatus, a paddle-beads method, was reported. With this method, some polystyrene beads were inserted into the dissolution medium to cause some frictional force or mechanical destruction of the dosage forms (Aoki et al 1992). Initially, we performed the dissolution study using the paddle-beads method. However, reliable results were not obtained, because the PCDC formulation was disintegrated by direct contact with the rotating paddle. To overcome this shortcoming, we tried to maintain the PCDC formulation at a fixed position between the paddle and the dissolution vessel wall. In this case, the frictional force was not transmitted to the PCDC formulation when the rotating speed was below $100\ rev\ min^{-1}$. By increasing the rotating speed to more than $100\ rev\ min^{-1}$, the frictional force was transmitted to the PCDCs and they disintegrated in the apparatus. To overcome the problems of the need for enough water and the transmittance of the frictional force in the conventional dissolution apparatus, we have proposed a rotating beads method for the dissolution test of the PCDC formulation. In this study, the difference in the drug dissolution rates between PCDC formulation and enteric tablet has been determined. However, the application of this method to colon delivery systems based on other delivery mechanisms such as time-controlled dissolution and microbially-controlled dissolution has not been elucidated. In particular, when this method is applied to microbially-controlled dissolution colon delivery systems, the luminal contents of the colon must be added to the dissolution medium.

References

Aoki, S., Uesugi, K., Ozawa, H., Kayano, M. (1992) Evaluation of the correlation between in vivo and in vitro release of phenylpropanolamine HCl from controlled-release tablets. Int. J. Pharm. 85: 65–73

Ashford, M., Fell, J. T. (1994) Targeting drugs to the colon: delivery systems for oral administration. J. Drug Target. 2: 241–258

Ashford, M., Fell, J. T., Attwood, D., Woodhead, P. P. (1993) An in vitro investigation into the suitability of pH-dependent polymers for colonic targeting. Int. J. Pharm. 91: 241–245

Digenis, G. A., Sandefer, E. (1991) Gamma scintigraphy and neutron activation techniques in the in vivo assessment of orally administered dosage forms. Crit. Rev. Ther. Drug Carrier Sys. 7: 309–345

El-Arini, S. K., Shiu, G. K., Skelly, J. P. (1990) Theophylline-controlled release preparations and fatty food: in vitro study using the rotating dialysis cell method. Pharm. Res. 7: 1134–1140

Evans, D. F., Pye, G., Bramley, R., Clark, A. G., Dyson, T. J., Hardcastle, J. D. (1986) Measurement of gastrointestinal pH profiles in normal ambulant human subjects. Gut 16:185–187

Gazzaniga, A., Iamartino, P., Maffione, G., Sangalli, M. E. (1994) Oral delayed-release systems for colonic specific delivery. Int. J. Pharm. 108: 77–83

Gazzaniga, A., Busetti, C., Moro, L., Sangalli, M. E., Giordano, F. (1995) Time-dependent oral delivery systems for colon targeting. S.T.P. Pharm. Sci. 5: 83–88

Gibaldi, M., Perrier, D. (1982) Pharmacokinetics. 2nd edn, Dekker, New York

Hardy, J. G., Davis, S. S., Wilson, C. G. (eds) (1989) Drug Delivery to the Gastrointestinal Tract. John Wiley, Chichester

Industrial Pharmacists Group (1991) Drugs and the gastrointestinal tract. Pharmaceut. J. 247: 137–139

Komuro, T., Yokota, C., Kimura, T. (1991) In-vitro dissolution properties of indomethacin extended-release capsules. J. Pharm. Pharmacol. 43: 79–82

Lui, C. Y., Amidon, G. L., Berardi, R. R., Fieisher, D., Youngberg, C., Dressman, J. B. (1986) Comparison of gastrointestinal pH in dogs and humans: implications on the use of the beagle dog as a model for oral absorption in humans. J. Pharm. Sci. 75: 271–274

Möse, A. J. (1993) Gastroretentive dosage forms. Crit. Rev. Ther. Drug Carrier Sys. 10: 143–195

Murata, S., Ueda, S., Shimojo, F., Tokunaga, Y., Hata, T., Ohnishi, N. (1998) In vivo performance of time-controlled explosion system (TES) in GI physiology regulated dogs. Int. J. Pharm. 161: 161–168

Narisawa, S., Nagata, M., Ito, T., Yoshino, H., Hirakawa, Y., Noda, K. (1995) Drug release behavior in gastrointestinal tract of beagle dogs from multiple unit type rate-controlled or time-controlled release preparations coated with insoluble polymer-based film. J. Contr. Rel. 33: 253–260

Olsson, B., Wagner, Z. G., Månsson, P., Ragnarsson, G. (1995) A gamma scintigraphic study of the absorption of morphine from controlled-release tablet. Int. J. Pharm. 119: 223–229

Ritschel, W. A. (1991) Targeting in the gastrointestinal tract: new approaches. Methods Find. Exp. Clin. Pharmacol. 13: 313–336

Röhm Pharm (1995) Information Sheets. Eudragit polymethacrylate for pharmaceutical applications.

Rubinstein, A., Tirosh, B., Baluom, M., Nassar, T., David, A., Radai, R., Gliko-Kabir, I., Friedman, M. (1997) The rationale for peptide drug delivery to the colon and the potential of polymeric carriers as effective tools. J. Contr. Rel. 46: 59–73

Saffran, M., Kumar, G. S., Savariar, C., Burnham, J. C., William, F., Neckevs, D. C. (1986) A new approach to the oral administration of insulin and other peptide drugs. Science 233: 1081–1084

Sako, K., Mizumoto, T., Kajiyama, A., Ohmura, T. (1996) Influence of physical factors in gastrointestinal tract on acetaminophen release from controlled-release tablets in fasted dogs. Int. J. Pharm. 137: 225–232

Sayani, A. P., Chein, Y. W. (1996) Systemic delivery of peptides and proteins across absorptive mucosae. Crit. Rev. Ther. Drug Carrier Syst. 13: 85–184

Shin-Etsu Chemical (1998) Information Sheets. Shin-Etsu POVAL (polyvinyl alcohol) for pharmaceutical applications.

Takaya, T., Ikeda, C., Imagawa, N., Niwa, K., Takada, K. (1995) Development of a colon delivery capsule and the pharmacological activity of recombinant human granulocyte colony-stimulating factor (rhG-CFS) in beagle dogs. J. Pharm. Pharmacol. 47: 474–478

Takaya, T., Sawada, K., Suzuki, H., Funaoka, A., Matsuda, K. Takada, K. (1997) Application of colon delivery capsule to 5-aminosalicylic acid and evaluation of the pharmacokinetic profile after oral administration to beagle dogs. J. Drug Target. 4: 271–276

Takaya, T., Niwa, K., Muraoka, M., Ogita, I., Nagai, N., Yano, R., Kimura, G., Yoshikawa, Y., Yoshikawa, H., Takada, K. (1998) Importance of dissolution process on systemic availability of drugs delivered by colon delivery system. J. Contr. Rel. 50: 111–122

Wakerly, Z., Fell, J., Attwood, D., Parkins, D. (1997) Studies on admidated pectins as potential carriers in colonic drug delivery. J. Pharm. Pharmacol. 49: 622–625

Zahirul, M., Khan, I. (1996) Dissolution testing for sustained or controlled release oral dosage forms and correlation with in vivo data: challenges and opportunities. Int. J. Pharm. 140: 131–143