

Characterization of Dual Layered Pellets for Sustained Release of Poorly Water-Soluble Drug

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The aim of this study was to develop pellet formulations that could be used to improve the dissolution and bioavailability of a poorly water-soluble model drug, cisapride. Six different types of pellets were prepared by coating sugar spheres in a fluidized bed coater. When the sugar spheres were single layered containing cisapride and solubilizer such as polysorbate 80, the resulting pellets provided an instant release of cisapride in the simulated gastric fluid. Dissolution tests carried out in the simulated intestinal fluid showed that there were negligible amounts of cisapride released, regardless of the pellet formulation. To succeed in attaining dissolution and the sustained release of cisapride at a neutral pH, the single layered pellets were coated again with a coating suspension containing Eudragit® RS 30D and L 30D. Scanning electron microscopy revealed that the dual layered pellets had a crack-free and spherical surface. Interestingly, the dual layered pellets provided the sustained release of cisapride in both the simulated gastric and intestinal fluids. The composition and components of the dual layers were found to be key parameters affecting the pattern of cisapride dissolution. Significant improvement in the bioavailability of cisapride was achieved when the dual layered pellets were administered orally to dogs. Overall, these results suggest that the dual layered pellets have potential as a sustained release dosage form for poorly water-soluble drugs.

Key words dual layered pellet; poorly water-soluble drug; solubilizer; sustained release; pharmacokinetics

Sugar spheres are inert excipients that can be coated with a drug-containing suspension and/or solution. They are useful in preparing pellets with a uniform drug content as well as for producing a modified release solid oral dosage form.^{1–3} Even though a variety of polymeric coating techniques have been developed, one popular method relies on the use of a fluidized bed coater. Several studies have focused on the critical process variables as well as their effects on the characteristics of the pellets.^{4,5} However, one limitation associated with a film, particulate and/or pellet coating is the failure to tailor the release patterns of poorly water-soluble drugs due to their limited aqueous solubility.⁶ Therefore, a variety of solubilizers including polyethylene glycol and surfactants have been used to improve the aqueous solubility before the coating process.^{7,8}

Cisapride has been used to treat adult patients with nocturnal heartburn due to gastroesophageal reflux disease but safety issues have restricted its use in many countries. However, it is still used widely to treat motility disorders in small animals such as dogs and cats. In this study, we prepared and optimized cisapride-loaded sugar spherical pellet formulations for sustained release. Cisapride was chosen as a model compound, on account of its peculiar solubility behavior: it is slightly soluble at an acidic pH but virtually insoluble in water and at a physiological pH. It was expected that a combination of solubilization and polymeric coating techniques could modify the release pattern of cisapride. In order to achieve these objectives, cisapride-loaded pellets were prepared with different formulations. Their dissolution patterns were investigated in the simulated gastric and intestinal fluids. The surface morphology and cross section of the dual drug-loaded pellets were also visualized using scanning electron microscopy (SEM). Finally, after administering the pellets orally to dogs, the pharmacokinetic parameters of the cisapride-loaded sugar spheres were compared with those of

a marketed tablet.

Experimental

Materials The acetone, oleic acid, polyethylene glycol 6000 (PEG6000), and polysorbate 80 were obtained from Showa Chemical Co. (Japan). The polyvinyl pyrrolidone (PVP) was purchased from Sigma-Aldrich (U.S.A.). Eudragit® RS 30D and the Eudragit® L 30D were kind gift from DucWoo Co. (Korea). The talc was obtained from Shinjun Pharmaceutical Co. (Korea). The cisapride was purchased from Morepen Laboratories Ltd. (India). The sugar spheres (mesh 18–20) were acquired from Crompton & Knowles Co. (U.S.A.).

HPLC Analysis of Cisapride Reverse phase HPLC was used to analyze the cisapride concentrations. The Jasco HPLC system used in this study consisted of a pump (Model PU-980), a UV detector (Model UV-975), an autosampler (Model AS-950-10), and an integrator (Borwin 1.20). A Crestpak C18 column (Jasco) was used as the stationary phase. A mixture of acetonitrile and 20 mM potassium dihydrogenphosphate (37:63, by v/v) was used as the mobile phase. The flow rate of the mobile phase was fixed to 1 ml/min. The elution of cisapride out of the column was detected at 275 nm. A series of known cisapride concentrations was used to produce a standard calibration curve based on the linear regression of their peak areas *versus* concentration.

Solubility Study An excess of cisapride was added to 4 ml of the simulated gastric or intestinal fluid as specified in the text. They were incubated at 25 °C for 3 d, and aliquots of their samples were filtered through a nylon membrane filter (0.45 µm pore size). The cisapride concentrations in the filtrates were determined by HPLC.

Preparation of Pellets with a Single Layer The active pellets were prepared by placing sugar spheres (100 g) in a fluidized bed coater (Model Strea 1; NiroAeromatic Inc., U.S.A.) and spraying a suspension and/or solution of the drug onto them. Table 1 shows the formulations used to prepare the various types of single layered pellets containing cisapride.

Prior to coating, the sugar spheres were prewarmed at 40 °C inside the fluid-bed coating chamber. For the production of batch 1, cisapride was first dissolved in acetone, and sprayed onto the sugar spheres. To prepare batch 2, a mixture of cisapride, polysorbate 80 and oleic acid was first heated at 80 °C followed by the addition of PEG6000. A cosolvent of acetone, water (1:5, by v/v) and the other excipients were added to the cooled mixture, and mixed well for 2 h. The coating suspensions were prepared in a similar manner for the production of batches 3, 4, and 5. Each coating solution/suspension was applied at to the sugar spheres a rate of 4 ml/min using a 0.8 mm nozzle with a continuous fluidizing air supply. The coating was carried out at

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Table 1. The Components and Composition Used to Produce the Various Types of Single Layered Pellets

Components (g)	Batch number				
	1	2	3	4	5
Cisapride	2.5	2.5	2.5	2.5	2.5
PVP	2.5	2.5	—	—	—
Polysorbate 80	—	2.5	2.5	2.5	2.5
Oleic acid	—	2.5	2.5	2.5	2.5
PEG6000	—	22.5	—	22.5	—
Eudragit® RS 30D	—	—	50	50	25
Eudragit® L 30D	—	—	—	—	25
Talc	5	5	3	3	3
Sugar spheres	100	100	100	100	100

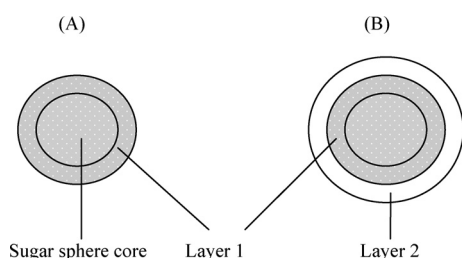


Fig. 1. Diagrams of the Single and Dual Layered Pellets

(A) represents a single layered pellet (batches 1—5), whereas (B) stands for a dual layered one (batch 6).

40 °C, and the atomizing air pressure was set to 1.6 bar, according to the typical processing conditions described elsewhere.^{5,9,10} After the coating was complete, the pellets were dried to a constant weight. In particular, the batch 2 pellets were subjected to further processing as described below.

Preparation of the Pellets with Dual Coating Layers One hundred grams of the batch 2 pellets were placed inside a fluid-bed coater and coated again using the coating suspension used for the production of batch 5. The resulting pellets were termed batch 6. In this study, batches 1—5 represent the single layered pellets, whereas batch 6 is the dual layered one. Figure 1 shows schematic diagrams of single- and dual-layered pellets.

Determination of Cisapride Contents in Coated Sugar Spheres Five hundred milligrams of the cisapride-loaded pellets were placed into a flask containing 50 ml acetone. Fifty milliliters of a 0.1 N-HCl solution was then added to this flask. The resulting suspension was centrifuged at 3500 rpm for 5 min. Aliquots of the supernatant were diluted with 0.1 N-HCl, and the cisapride content was determined by HPLC.

Determination of Cisapride Release from Pellets *In vitro* dissolution tests were carried out on various types of cisapride-loaded pellets equivalent to 10 mg doses using the USP Apparatus I (the basket method at 100 rpm; Model DST-600A; Fine Lab, Korea). The following dissolution media were used in this study: 500 ml of the simulated gastric fluid (0.1 N HCl-NaCl) and the simulated intestinal fluid (50 mM phosphate buffer at pH 6.8). The temperature was maintained at 37±0.5 °C during the dissolution study. At predetermined time intervals, aliquots (1 ml) of the dissolution media were withdrawn, and the cisapride concentrations were determined by HPLC.

Scanning Electron Microscopy (SEM) of Pellets The dried samples were coated with gold using a JEC-1100 sputter coater (Jeol Korea Ltd., Korea) and then examined by SEM (Model CHK 1M0083; Olympus Corp., Japan).

Dog Experiments A pharmacokinetic study of the batch 6 formulation in comparison with a marketed tablet was carried out in dogs. The batch 6 pellets (equivalent to 10 mg of cisapride) were filled into a 0-sized hard gelatin capsule. Four male beagle dogs (10.0—13.0 kg) were housed individually inside stainless steel cages and fasted for 12 h before administering the drug. The dogs were orally administered the capsule and the tablet in a crossway design. Blood samples (7 ml) were withdrawn from the jugular vein with heparinized syringes at predetermined time intervals. The dogs were allowed to eat food after 3 h of dosing. The plasma samples were prepared by centrifugation and stored at -70 °C until assayed.

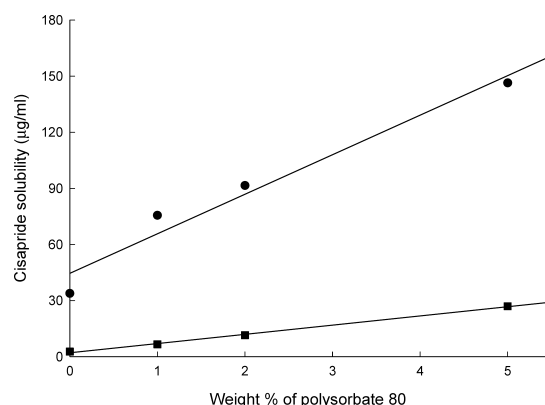


Fig. 2. Effect of Polysorbate 80 upon Cisapride Solubility

The degree of cisapride solubility in (●) the simulated gastric and (■) intestinal fluids increased linearly as a function of the polysorbate 80 concentration.

Determination of Plasma Concentration of Cisapride The plasma samples were spiked with the internal standard solution (ethyl paraben in ethanol), mixed and shaken with 6 ml of *t*-butyl methyl ether. After centrifugation, the upper organic solvent layer was withdrawn and transferred to other test tubes. The organic solvent was evaporated under a hot air stream, and the residue was reconstituted with 200 µl of the HPLC mobile phase described earlier. The cisapride concentrations were determined by HPLC.

Results and Discussion

The solubility of cisapride in the simulated gastric fluid, the simulated intestinal fluid and water was 33.8 µg/ml, 4.0 µg/ml and 2.7 µg/ml, respectively. It was possible to increase the magnitude of its aqueous solubility using polysorbate 80 (Fig. 2). The so-called micellar solubilization process helped improve its aqueous solubility. It is known that micelles can have a variety of morphology, depending upon surfactant concentration but this aspect is beyond the scope of our present study. There was a linear increase in the cisapride solubility in the simulated gastric and the simulated intestinal fluids. Based on these results, solubilizing excipients such as polysorbate 80 were added to most of the pellet formulations. However, dissolution of drug by itself is hardly occurred in the simulated intestinal fluid. For this reason, other excipients like oleic acid, PVP, PEG6000 and other Eudragit polymers were properly used in the formulations to increase dissolution rate of cisapride in a sustained manner without showing any supersaturation of drug.

Figure 3 shows the actual cisapride content present in the various types of pellet formulations. The amounts of cisapride found in batches 2, 3, 4 and 5 were comparable to each other. When only cisapride was dissolved in acetone and sprayed onto the sugar spheres, the amount of cisapride found in the sugar spheres was 4.7±3.2 mg/g (mean±S.D.). In contrast, the inclusion of several excipients such as polysorbate 80, oleic acid and PEG6000 in pellet formulations significantly improved the level of cisapride deposition onto the sugar spheres. For example, after the coating was carried out to make the batch 2 pellets, 21.2±3.4 mg of cisapride per gram of sugar spheres was layered. Similar results were attained with batches 3, 4 and 5, and there were no significant differences between the batches. This indicates that the coating process reported in this study is reproducible and likely to have practical applications.

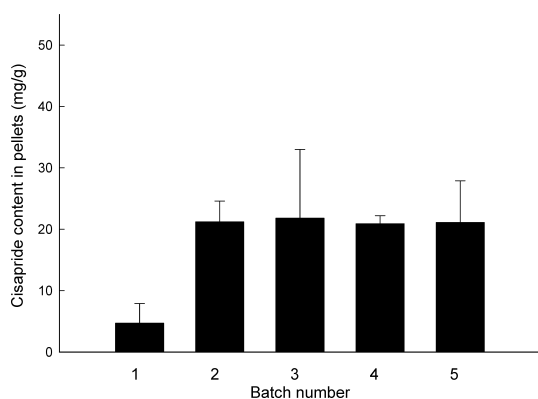


Fig. 3. The Actual Content of Cisapride Found in Various Pellet Formulations

The amount of cisapride found in batches 2, 3, 4 and 5 were comparable to each other.

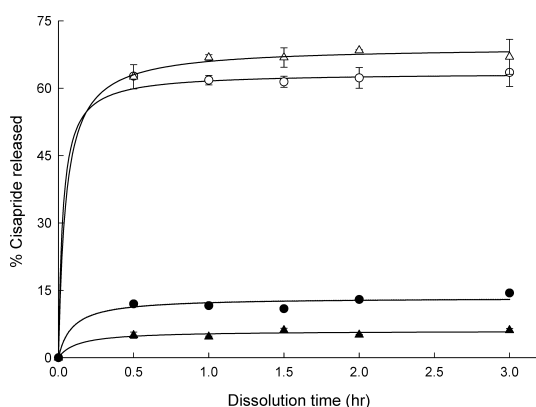


Fig. 4. Release Profiles Attained with the Single Layered Pellets of Batch 2 (○, ●) and a Marketed Product (△, ▲)

Dissolution tests were carried out using (open symbols) the simulated gastric and (filled symbols) intestinal fluid.

The dissolution test on the batch 2 pellets demonstrated that they provided the instant release of cisapride: when the simulated gastric fluid was used as a dissolution medium, $62.7 \pm 0.5\%$ of cisapride was released within 30 min (Fig. 4). However, when the simulated intestinal fluid was used as the dissolution medium, the amount of cisapride released in 30 min was only $11.9 \pm 1.8\%$. From a marketed product, cisapride released $62.6 \pm 2.7\%$ and $5.0 \pm 0.7\%$ within 30 min at the simulated gastric fluid and the intestinal fluid, respectively. The batch 2 pellets showed similar dissolution with the marketed product at the simulated gastric fluid and increased a little the dissolution at the simulated intestinal fluid. Prolonging the dissolution test up to 3 h did not facilitate the release of cisapride. This demonstrates that the batch 2 pellets do not provide the controlled release of cisapride in the simulated gastric fluid. Furthermore, its negligible solubility at a neutral pH might be responsible for the poor dissolution in the simulated intestinal fluid.

Figure 5 shows the release patterns attained with the different single layered pellet formulations (batches 3, 4, 5). The amount of cisapride released in the simulated gastric fluid from batch 3 was significantly lower than that observed with the batch 2 pellets. Indeed, only $31.6 \pm 0.9\%$ of cisapride was released after the dissolution test for 5 h. It appears that

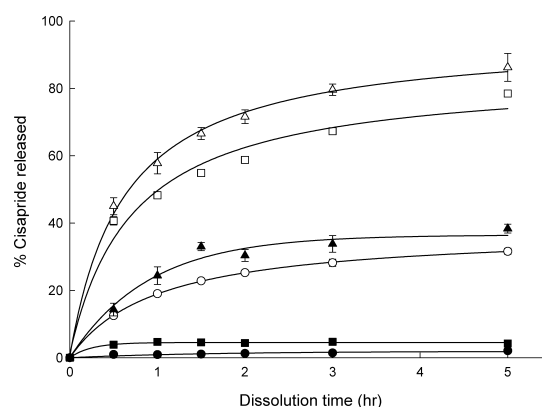


Fig. 5. Release Profiles Observed with the Single Layered Pellets of Batches (○, ●) 3, (□, ■) 4, and (△, ▲) 5

Dissolution tests were conducted using (open symbols) the simulated gastric and (filled symbols) intestinal fluids.

Eudragit® RS 30D, which is a pH independent polymer commonly used in sustained release formulation, slows down the diffusion of cisapride across the single layered pellets. In contrast, as observed with batches 4 and 5, the addition of either PEG6000 or Eudragit® L 30D to the batch 3 formulation led to the pronounced enhancement in the amounts of cisapride released. Another advantage of batches 4 and 5 on the batch 3 pellets is that they provide the sustained release of cisapride in gastric fluid. PEG6000 appears to have an enhancing effect on cisapride dissolution. Eudragit® L 30D, which is a pH-dependent polymer soluble above pH 5.5, is an enteric polymer that is currently used to coat pharmaceutical dosage forms.¹¹ These results show that blending Eudragit® L 30D and RS 30D at a suitable ratio is a useful approach to control the release pattern of cisapride. This suggestion is in line with a recent report showing that Eudragit-based formulations help prepare the prolonged-release diclofenac microcapsules.¹²

Unfortunately, none of the batches attained the sustained release of cisapride in the simulated intestinal fluid. In an attempt to control the release of cisapride at pH 6.8, another polymeric layer (batch 5) was applied to the batch 2 pellets to produce batch 6. Figure 6 shows the external and internal morphology of the dual drug-loaded pellets (batch 6). The dual layered pellets had a spherical and smooth surface. Cracks or holes were absent on their surface, and two distinct layers could be seen in the cross-section. Figure 7 shows the dissolution patterns of the dual layered pellets of batch 6. The batch 6 pellets with the dual polymeric layers provided the sustained release of cisapride in both the simulated gastric and the simulated intestinal fluids. The batch 6 is clearly distinct from the batch 5 in that the former has another layer containing PEG6000 and PVP. Our results demonstrate that the layer also plays a vital role in the sustained release of cisapride in the simulated intestinal fluid. Their dissolution-enhancing effects could be attributed to the facts that PEG6000 and PVP would contribute to forming interstitial solid solutions with cisapride, reducing drug particle aggregation, and/or altering surface properties of drug particles, as suggested elsewhere.^{8,13} This would be absolutely true in the intestinal fluid if the dissolution medium maintains a sink condition. However, the solubilization capacity of single layered

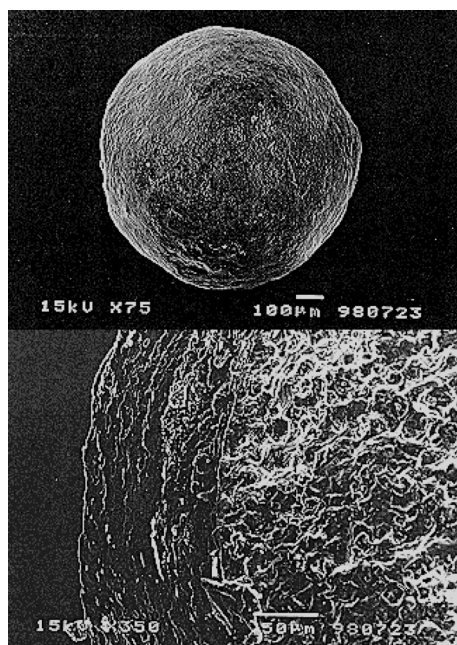


Fig. 6. SEM Micrographs of Dual Layered Pellets (Batch 6)

Their surface is crack/hole-free and spherical. Two distinct layers are clearly demonstrated.

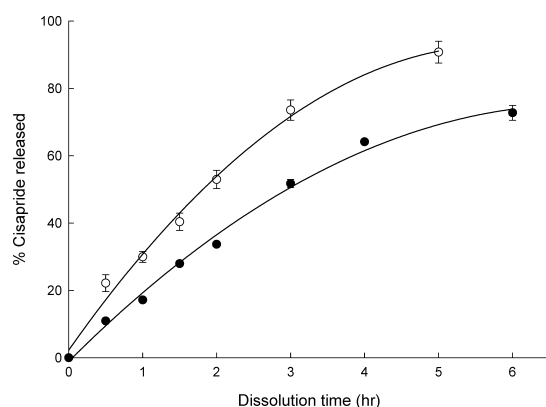


Fig. 7. Dissolution Profiles Attained with the Dual Layered Pellets of Batch 6

Dissolution tests were conducted using (○) the simulated gastric and (●) intestinal fluids.

pellet formulations was not satisfactory. Contrarily, the batch 6 formulation also contains more solubilizers (*e.g.*, polysorbate 80 and oleic acid) to improve cisapride solubility. These distinct features are thought to be responsible for the increased cisapride dissolution of batch 6 pellets in a sustained manner for 6 h without showing any precipitation. Meanwhile, Eudragit® L 30D and RS 30D appear to serve as effective matrices allowing the sustained release of cisapride in the simulated gastric fluid. The acceleration of the dissolution rate of cisapride in the simulated intestinal fluid might be attributed to Eudragit® L 30D, which dissolves at $\text{pH} \geq 5.5$. The rationale for choosing Eudragit RS 30D and L 30D comes from our formulation strategy. Eudragit RS 30D is a pH-independent polymer frequently used in the sustained release formulation. If combined with Eudragit L 30D which dissolves at $\text{pH} \geq 5.5$, the release of cisapride from our dual layered pellets would be facilitated at the intestinal tract. The

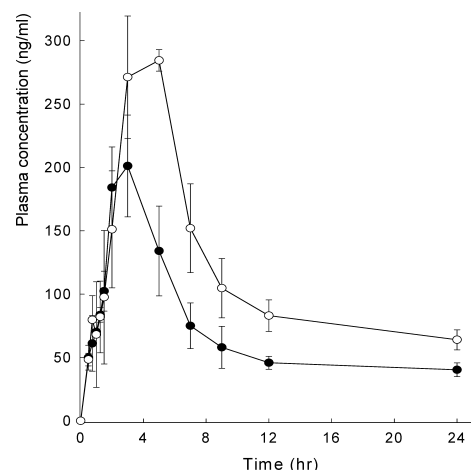


Fig. 8. Plasma Concentration–Time Curves Observed after a Single Oral Dose of 10 mg Cisapride in (○) the Dual Layered Pellets and (●) the Tablet ($n=4$)

The dual layered pellet dosage form helps improve the bioavailability of cisapride.

Table 2. The Pharmacokinetic Parameters of Cisapride after Oral Administration in Four Dogs

PK parameter	The marketed product	The dual layered pellets
$C_{\max}$ (ng/ml)	209.68 ± 0.71	295.41 ± 24.51
$T_{\max}$ (h)	2.50 ± 0.71	4.00 ± 1.41
$AUC_{0 \rightarrow 24 \text{ h}}$ ($\mu\text{g/ml} \cdot \text{h}$)	1.70 ± 0.06	2.77 ± 0.04
$AUC_{0 \rightarrow \infty}$ ($\mu\text{g/ml} \cdot \text{h}$)	3.54 ± 1.43	5.26 ± 1.22

results of our study prove that this strategy works well. In the future study, we plan to study thoroughly the effects of the characteristics of a second layer upon drug release profiles.

Figure 8 shows the plasma concentration–time curves of the batch 6 dosage form and a marketed tablet. The parameters were calculated using WinNonlin® and statistically evaluated with *t*-test. The $C_{\max}$, $T_{\max}$, $AUC_{0 \rightarrow 24 \text{ h}}$ and $AUC_{0 \rightarrow \infty}$ of the dual layered sugar spheres were $295.41 \pm 0.71 \text{ ng/ml}$, $4.00 \pm 1.41 \text{ h}$, $2.77 \pm 0.04 \text{ h} \cdot \mu\text{g/ml}$, and $5.26 \pm 1.22 \text{ h} \cdot \mu\text{g/ml}$, respectively (Table 2). The curve pattern of a marketed tablet was similar to that of the batch 6 dosage form. However, the values of the pharmacokinetic parameters of the dual layered pellets were 1.4–1.6 times higher than those of the marked tablet ($p < 0.05$). Dosage forms must release a drug substance in the solution while they pass through the gastrointestinal tract. At this time, a number of poorly water-soluble drugs (*e.g.*, BCS class II drugs) are subject to solubility-limited absorption. It has been speculated that the enhancement of cisapride dissolution in the gastric and intestinal fluids might contribute to improving its bioavailability. Our result is consistent with the view that enhancing the solubility of poorly water-soluble drugs often enhances their bioavailability.^{14,15)}

Conclusions

The dual layered pellets with an optimal formulation provided the sustained releases of a poorly water-soluble model drug, cisapride, in both the simulated gastric and intestinal fluids. Its enhanced dissolution is essential for making a sustained release dosage form. In addition, the release pattern of cisapride can be manipulated by blending it with release-

modifying polymers of Eudragit® RS 30D and L 30D at a suitable ratio. Indeed, the dual layered pellets were found to increase the bioavailability of cisapride.

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