

Design and *in Vitro/in Vivo* Evaluation of Multi-layer Film Coated Pellets for Omeprazole

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Received May 29, 2008; accepted November 18, 2008; published online November 21, 2008

The purpose of the study is to perform the *in vitro* and *in vivo* evaluation of multi-layer film coatings for omeprazole. The system consists of drug-layered or drug-containing core pellets coated with salt (sodium chloride and disodium hydrogen phosphate), hydroxypropyl methyl cellulose (HPMC), and enteric film-coating layer, respectively. The drug-layered core pellets were prepared by a coating layer of omeprazole on inert pellet cores in fluidized bed coater. An *in vitro/in vivo* gastro-resistance study was conducted, and a dissolution study was performed in pH 7.4 phosphate buffer for omeprazole release. The multi-layer coated pellets were stable in gastric pH conditions and upper gastrointestinal (GI) tract in rats. Salt layer improved the drug stability, and its coating levels had little influence on the dissolution profiles of omeprazole. The rate of drug release was significantly delayed by HPMC layer. The salt layer could function as a separated layer, and substitute part of the HPMC layer and decrease the coating levels of HPMC. The bioavailability (*AUC*) of the multi-layer coated drug-layered and drug-containing pellets was 3.48 ± 0.86 and $2.97 \pm 0.57 \mu\text{g} \cdot \text{h}/\text{ml}$, respectively. The drug-layered pellets with multi-layer film coatings not only provided delayed and rapid release of omeprazole, but also could provide a good stable property for omeprazole. It was confirmed that rapid *in vitro* drug release rate resulted in better absorption.

Key words film coating; omeprazole; pharmacokinetics; Eudragit[®] L 30D-55

Proton-pump inhibitors (PPIs) have been very efficacious for the management of a variety of acid-related disorders. However, as PPIs are acid-labile, they need to be protected from the destructive effects of gastric acid when administered orally.¹⁾ Most PPI absorption takes place in the proximal small intestine.

Omeprazole, 5-methoxy-2-[[[4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulphonyl]-1H-benzimidazole, a substituted benzimidazole compound and prototype anti-secretory agent, is the first of the “proton pump inhibitors” widely used for the prophylaxis and treatment of gastro-duodenal ulcers and for the treatment of symptomatic gastro-oesophageal reflux. It acts by interaction with H^+/K^+ ATPase in the secretory membranes of the parietal cells and is very effective in the treatment of Zollinger–Ellison syndrome. Although its elimination half-life from plasma is short, reported to be about 0.5–3 h, its duration of action with regard to inhibition of acid secretion is much longer allowing it to be used in single daily dose.²⁾

Omeprazole is a lipophilic, weak base with $\text{p}K_{\text{a}1}=4.2$ and $\text{p}K_{\text{a}2}=9$.³⁾ It is a poorly water-soluble white powder, and degrades rapidly in aqueous solutions at low pH values.^{3,4)} It has a very short half-life of 10 min at pH values below 4, but 18 h at pH 6–8, and about 300 d at pH 11.^{5,6)} It was reported that moisture, heat, solvents, and acidic substances had deleterious effects on the stability of omeprazole.⁷⁾ In addition, other investigators had observed a degradation of omeprazole under exposure to UV light, and some metal ions.^{3,4,8)} Discoloration ranging from light beige to deep purple will occur immediately when omeprazole is exposed to unfavorable conditions. To overcome the stability problems of omepra-

zole, the best approach appears to be the application of an enteric coating onto the solid dosage form. However, enteric polymers are polymeric acids with free carboxyl groups. Therefore, it could be expected that the stability of omeprazole was affected by the polymeric acids used to obtain gastric resistance.^{9–13)} The separating layer could serve as a pH-buffering zone if in contact with a core containing alkaline stabilizers, but it was reported that even hydroxypropyl methyl cellulose (HPMC) appeared to interact with the coating material and therefore could not be regarded as an inert material.¹⁴⁾ It was also reported that more than half of commercial omeprazole products could not maintain the required stability,⁷⁾ and that bioavailability might be affected by the poor dissolution of omeprazole from the dosage form.

An enteric coated dosage form of omeprazole was reported by Pilbant and Cederberg.⁵⁾ The publication describes a conventional enteric coated dosage form and states that it has acceptable storage stability-for clinical studies. However, it was later found that the stability of this dosage form was insufficient during long-term storage required for a marketed pharmaceutical dosage form. In addition, to overcome the stability and solubility problems of omeprazole, methods of preparing cyclodextrin inclusion complexes^{15–18)} and tableting enteric-coating pellets¹⁹⁾ have also been tried.

The purpose of the study is to perform the *in vitro/in vivo* evaluation of multi-layer film coatings for omeprazole. The multi-layer coated system consists of drug-layer cores/drug-containing pellets, coated with a salt, a HPMC layer and an enteric layer, respectively.

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Experimental

Materials The following materials were obtained from the indicated sources. Omeprazole used in this study was purchased from Changzhou No. 4 Pharmaceutical Ltd. (Changzhou, China). The pellet cores consisted of microcrystalline cellulose (MCC) as an extrusion aid (Avicel PH101, Shandong, China). Lactose (Shengli Ltd., Hangzhou, China) and Mannitol (BASF, Ludwigshafen, Germany) were used as components of the cores pellets. Eudragit L30D-55 plasticized with PEG 6000 (Merck, Germany) was a gift from Röhm Pharma (Darmstadt, Germany). Hydroxypropylmethylcellulose 60RT5 (HPMC, 60RT5) was purchased from Feichengruitai Ltd. (Shandong, China). All chemicals were of analytical grade.

Preparation of Drug-Containing and Drug-Layered Pellet Cores Pellet cores were prepared by the process of extrusion-spheronisation (E-extrusion granulator, R-spheronizator, Research Institute of Process Equipment and Pressure Vessels, East China University of Science, Shanghai, China), which travelled at 50 mm/min and spheronised for 5 min at 500 rpm. Those pellets in the size fraction 0.8–1.0 mm (greater than 65% yield in this size range) were used in subsequent studies. The drug-containing pellet cores consist of a drug omeprazole, 20% MCC, 2% sodium carbonate (Na_2CO_3), and 2% sodium dodecyl sulfate (SDS), 20% lactose and 50% mannitol. The inert pellet cores consist of 10% MCC, 80% mannitol and 10% lactose. Distilled water was used as granulation liquid. The pellets were dried for 6 h at 35–40 °C.

The drug-layered pellet cores were prepared by spraying omeprazole onto inert cores (0.5–0.8 mm) in fluidized bed coater (Jiafa Granulating drying equipment, Changzhou, China) to achieve a 15% (w/w) drug content under the same conditions as described below. The active omeprazole layer surrounds an inert core. The composition of omeprazole layer was given as follows: omeprazole, 40 g; MgO, 10 g; SDS, 5 g; 0.01 M NaOH, 400 g. PEG 6000 (15 g), PVPK30 (15 g) or mannitol (10 g) were used as a binder.

Coating of the Pellet Cores The drug-containing and drug-layered core pellets were coated with three successive layers: an inner salt, a HPMC and an enteric-coating layer, respectively. The film thickness was expressed as the theoretical percentage of the weight gained TWG (%).

The composition of salt layer was listed as follows: NaCl, 60 g; Na_2HPO_4 , 40 g; Na_2CO_3 , 20 g and 0.01 M NaOH 400 g. PVPK30 (15 g) was added as a binder. The solid content was 34.2%. The coating level of salt layer was 15 to 35% (w/w).

The HPMC layer was 3% (w/w) HPMC solution plasticized with PEG 6000 (10% (w/w) based on the solid content of HPMC). Titanium dioxide (TiO_2 , 5% (w/w) based on polymer solids) was added into the solution. The coating levels of HPMC layer were 15 to 35% (w/w).

The two-layer coated pellets were subsequently coated with aqueous methacrylic acid copolymer dispersion (Eudragit® L 30D-55) to achieve a weight gain of 20 to 35% (w/w) to obtain the complete multi-layer coated pellets (Fig. 1). A plasticizer (PEG 6000; 10% (w/w) based on polymer solids) and titanium dioxide (TiO_2 , 5% (w/w) based on polymer solids) was added into the methacrylic acid copolymer dispersions and the dispersions were gently stirred for at least 30 min prior to an appropriate dilution with purified water and subsequent coating. The solid content of the coating dispersions was 15% (w/w).

The coating conditions for drug layer, salt layer, HPMC layer and enteric layer in fluidized bed coating apparatus were listed as follows: batch size (g), 240; nozzle bore (mm), 1.0; atomizing pressure (bar), 0.8–1.0; inlet air temperature (°C), 45–50; inlet air (m^3/h), 45–50; exhaust air temperature (°C), 30–35; pellet bed temperature (°C), 25–30; spray rate (ml/min), 2.0; drying in the equipment after coating (min), 15; final drying in oven, 24 h, 40 °C.

In Vitro Drug Release Studies Dissolution test was conducted in USP apparatus 1 (Tianda Tianfa Technology Co., Ltd., Tianji, China) at 100 rpm and a temperature of 37.5 ± 0.5 °C. A gastro-resistance study was conducted in 750 ml of 0.1 M HCl (pH 1.2) for 2 h. A dissolution study was performed in pH 7.4 phosphate buffer for omeprazole release. A 2 ml sample was withdrawn from each dissolution beaker after regular intervals of time and then 0.5 ml of 0.25 M NaOH solutions was added. The samples were filtered through a 4.5 μm filter and injected into the HPLC system as described below.

Stability Studies To assess the long-term stability, multi-layer coated drug-layered and drug-containing pellets (The coating levels for salt, HPMC and enteric layer were TWG=35%, respectively.) were stored at 40 °C/75% relative humidity (RH) for 6 months. After the first, second, third, fourth, fifth and the sixth months, the two formulations were observed for change in physical appearance, color, drug content and drug release characteristics.

In Vivo Gastro-resistance and Stability in Upper GI Tract for Multi-

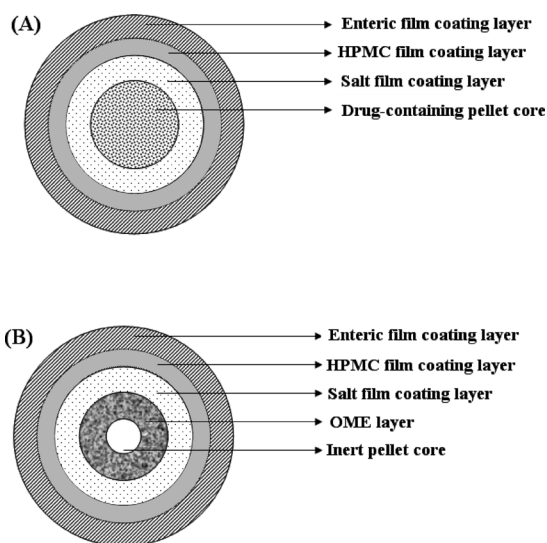


Fig. 1. Schematic of the Multi-layer Film Coated Pellet of Omeprazole (Not to Scale)

(A) Multi-layer film coated drug-containing pellet, (B) multi-layer film coated drug-layered pellet.

layer Film Coated Pellets In order to evaluate the *in vivo* gastro-resistance and stability in upper GI tract, six rats (Experimental animals' center of Hebei Medical University, China) weighing 150–200 g were used. The rats were divided randomly into two groups and fasted for 12 h. Multi-layer coated pellets (The coating levels for the salt, HPMC and enteric layer was TWG=35%, respectively.) were administered to the rats *via* a polyethylene cannula (diameter: 2 mm) with 1 ml water under light ether anesthesia, respectively, at a dosage of 15 mg/kg. At specific time points (0.25, 0.5, 1.0, 1.5, 2.0 h) after administration, the rats were anesthetized by halothane and killed. The coated pellets were collected from the gastro and small intestine. The collected pellets were washed with distilled water, and the surface of the coated pellets was observed.

Pharmacokinetics in Dogs The pharmacokinetics of reference (Losec®), multi-layer coated drug-layered pellets (Formulation A, The coating levels for salt, HPMC and enteric layer were TWG=35%, respectively.) and multi-layer coated drug-containing pellets (Formulation B, The coating levels for salt, HPMC and enteric layer were TWG=35%, respectively.) assessed and compared in beagle dogs in a three formulation, randomized, three-period crossover study. The washout period between administrations was one week. Six male beagle dogs (Experimental animals' center of Hebei Medical University, China) weighing from 8 to 10 kg were used in this study. The dogs were fed standard laboratory chow with water and fasted overnight before the experiments. The animals used in the experiments received care in compliance with the "Principles of Laboratory Animal Care" and "Guide for the Care and Use of Laboratory Animals." Experiments followed an approved protocol from Hebei Medical University Institutional Animal Care and Use Committee.

The multi-layer coated pellets were filled into a hard gelatin capsule, and orally administered in beagle dogs at a dosage equivalent to 3.0 mg/kg. All of the formulations were administered with 20 ml of water. At time intervals, 2 ml of blood samples were collected from saphenous vein into heparinized tubes and centrifuged at $4000 \times g$ for 10 min and stored at -20 °C until assay. Blood samples were collected from the saphenous vein at 0, 0.5, 1.0, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 4.0, 5.0 and 6.0 h.

Frozen plasma samples were thawed; an aliquot (0.5 ml) of plasma sample was measured into a glass tube with a teflon-lined cap, followed by the addition of 0.05 ml of methanol: acetate buffer (pH=9.6) (1:4, v/v). After that, 2.5 ml of dichloromethane: acetonitrile (4:1, v/v) was added and vortexed for 1 min. Following centrifugation at $3000 \times g$ for 10 min, 2 ml of the organic phase was separated and evaporated under a nitrogen stream. The residue was dissolved in 100 μl of mobile phase, and 20 μl was subjected to HPLC analysis of omeprazole under conditions as described below.

HPLC Analysis Concentrations of omeprazole in plasma samples were determined by HPLC. Torasemide was used as an internal standard.²⁰ The HPLC system consisted of a Waters 2487 UV detector (Water Assoc., Milford, MA, U.S.A.) and an Empower workstation (Water Assoc., Milford,

MA, U.S.A.). Separations were performed at 25 °C using a 250 mm×4.6 mm column (Diamonsil C18, Dikma, U.S.A.). The mobile phase was consisted of methanol/water/triethylamine/phosphoric acid (67/33/0.3/0.12, v/v), which was filtered and delivered at a flow rate of 1 ml/min. The column was maintained at a temperature of 25 °C. The eluent was detected by UV detector at 302 nm. The retention times of omeprazole and an internal standard were approximately 9.6 and 7.6 min, respectively. The detection limit of omeprazole in dog plasma was 15 ng/ml. The intra-day and inter-day variation of the HPLC method were found to be less than 4.3% (CV) and less than 8.2% (CV), respectively.

Data Analysis Pharmacokinetic (PK) parameters were calculated by non-compartment analysis based on statistical moment theory using Microsoft Excel software. C_{max} and T_{max} were obtained directly from the plasma concentration–time plots. The area under the plasma concentration–time curve up to the last time (t) (AUC_{0-t}), area under curve extrapolated to infinity ($AUC_{0-\infty}$) and area under the first moment curve extrapolated to infinity ($AUMC_{0-\infty}$) were calculated using the linear trapezoidal rule. The mean residence time (MRT) was calculated as $AUMC/AUC$. The relative bioavailability (BA) was calculated using the formula: $100\% \times (AUC_{test}/AUC_{reference})$.

The observed variation in the pharmacokinetic parameters was tested by using analysis of variance (ANOVA). The observed difference in mean pharmacokinetic parameters of omeprazole from the multi-layer film coated pellets and the reference was subjected to t -test to find the statistical significance. In all the cases, a value of $p < 0.05$ was considered statistically significant.

Results and Discussion

Effect of Formulation Variables on Drug Release.

Preparation of Drug-Layered and Drug-Containing Core Pellets The percent of omeprazole released from the drug-layered and drug-containing core pellets is shown in Fig. 2. Compared with drug-containing pellets, the drug-layered pellets could provide a much more rapid drug-release rate ($p < 0.05$). Omeprazole is a poorly water-soluble drug; but approximately 100% omeprazole was released within 6 min from drug-layered pellets, while 45% omeprazole was released from drug-containing pellets at the same time. When the active drug layer surrounded the inert cores, the release rate could be significantly improved. In order to prepare the drug-layered pellets in the fluidized bed coater, three kinds of binders (PVPK30, mannitol and PEG 6000) were used. The kinds of binders had little influence on the dissolution profiles of omeprazole. Strictly considered, the dissolution rate of omeprazole from drug-layered pellets was in the order mannitol > PVPK30 > PEG 6000. Thus, mannitol was used as a binder in the preparation of drug-layered pellets in the further experiment.

The rate of drug release from drug-layered core pellets was much faster than that from drug-containing core pellets. It might be the reasons that omeprazole, which was completely dissolved in the alkaline solution in drug-layered pellet cores, existed in an amorphous form, which contributed to the enhanced dissolution rate.

Effect of the Coating Levels on the Drug Release. Effect of Salt Film Coatings In order to improve the stability of the omeprazole, the salt layer was coated on the drug-layered and drug-containing core pellets. Figure 3 demonstrates the effect of salt film coating levels on the drug release from the salt film coated pellets. The salt layer did not delay release of the omeprazole from the drug-layered and drug-containing-core pellets to any significant extent, and the dissolution rate was almost the same from the core pellets without salt layer. Furthermore, variation in coating levels had little influence on the dissolution profiles of omeprazole.

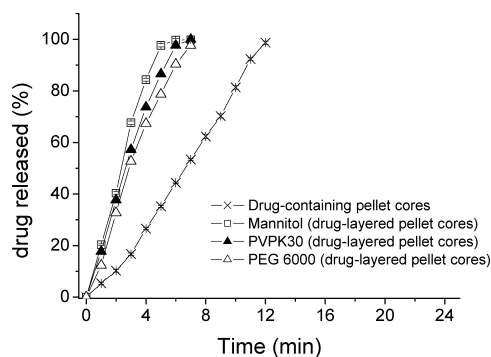


Fig. 2. *In Vitro* Dissolution Profiles for Drug Release from Drug-Containing Pellet Cores and Drug-Layered Pellet Cores (with Mannitol, PVPK30 and PEG 6000 as a Binder, Respectively) in pH 7.4 Phosphate Buffers

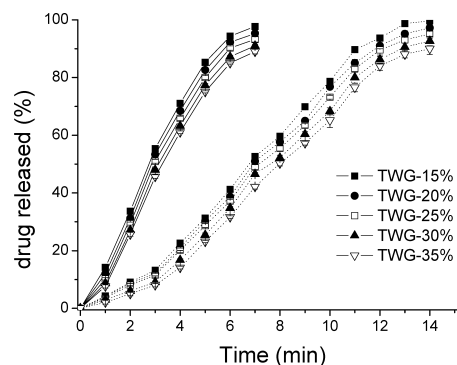


Fig. 3. Effect of Coating Level (%TWG) of Salt Layer on Drug Release from Pellet Cores in pH 7.4 Phosphate Buffer

Complete line=salt film coated drug-layered pellets, dotted line=salt film coated drug-containing pellets.

Effect of HPMC Film Coatings After coated with aqueous solution of sodium chloride and disodium hydrogen phosphate, the salt coated pellets, which the coating level for salt layer was TWG-35%, were subsequently coated with HPMC layer with different film thickness (TWG % of 15, 20, 25, 30, 35). The dissolution profiles from the two-layer film coated pellets are shown in Fig. 4. The release rate of omeprazole was significantly delayed by the HPMC film coating layer in the case of drug-layered pellets. The rate of drug release was inversely proportional to the thickness of the coat, suggesting that the HPMC layer would increase the diffusional path length between the pellet core and the dissolution medium. The rate of drug release from the two-layer film coated drug-layered pellets was much faster than that from two-layer film coated drug-containing pellets. When the HPMC film coating level was TWG=15%, the drug-layered pellets with salt/HPMC film coatings released about 98% of omeprazole within 15 min whereas the drug-containing pellets released only about 63% of omeprazole. It might be the reasons that the inert cores contained one osmotic agent (mannitol) that promoted a buildup of osmotic pressure in the core. When the drug-layered cores contacted with dissolution medium, the dissolution medium could penetrate into the core, and the drug-layer would function as an osmotic pressure-entrapped layer. When the osmotic pressure was accumulated to some extent, then the cores ruptured rapidly (about 2—3 min in our experiment). Additionally, surfactants (SDS) contained in the drug layer would also enhance the

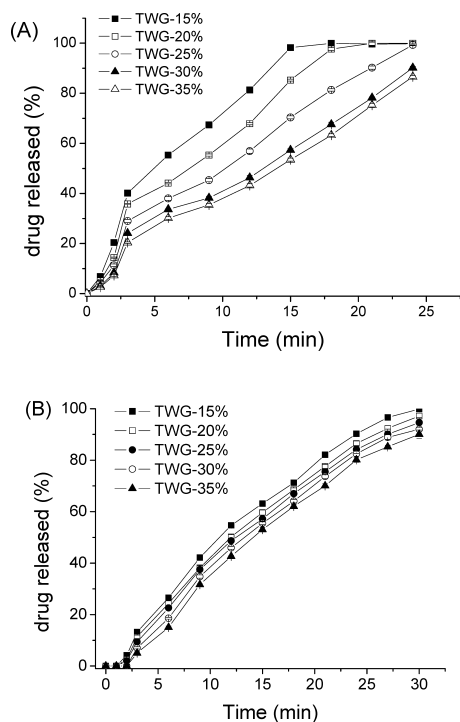


Fig. 4. Effect of Coating Level (%TWG) of HPMC Film Coating Layer on Drug Release from Salt Layer Coated Pellets (the Coating Level for Salt Layer Was TWG=35%) in pH 7.4 Phosphate Buffer

(A) Drug-layered pellet cores, (B) drug-containing pellet cores.

dissolution rate of omeprazole. Monnitol was also added in drug-containing cores, but the osmotic pressure was not accumulated since osmotic pressure-entrapped was absent. Thus, the drug-containing cores could not disintegrate rapidly within a short time (about 11 min), which resulted in relatively slower release. Hence, when the active omeprazole, a poorly water-soluble drug, surrounded the inert cores, its dissolution rate could be improved significantly.

The drug release from drug-layered pellets was significantly delayed by the HPMC film coating levels. If only the HPMC layer was used as separating coating, the coating levels must be high enough to prevent the drug migrating into the enteric layer; however, the thick coatings would delay drug release significantly. When the salt layer functioned as a separating coating, it would reduce the coating thickness of the HPMC layer, which would be beneficial to the rate of drug release. In addition, HPMC aqueous solutions have pH between 4 and 8, and omeprazole was a weak based drug. This indicated that HPMC was not beneficial to the stability of omeprazole during the coating process or during storage. Thus, the salt layer could not only separate the omeprazole loading core from the HPMC layer, but also did not delay the drug release.

Effect of Enteric Film Coatings It is well known that omeprazole is sensitive to acidic conditions and after contact with an acid; Omeprazole will degrade and will not function in its intended manner. Thus, the formulation should be enteric coated. The two-layer coated pellets, which the coating levels for both the salt and HPMC layers were TWG=35%, were subsequently coated with aqueous methacrylic acid copolymer dispersion (Eudragit® L 30D-55) at different coating levels to obtain the complete enteric coated pellets. The

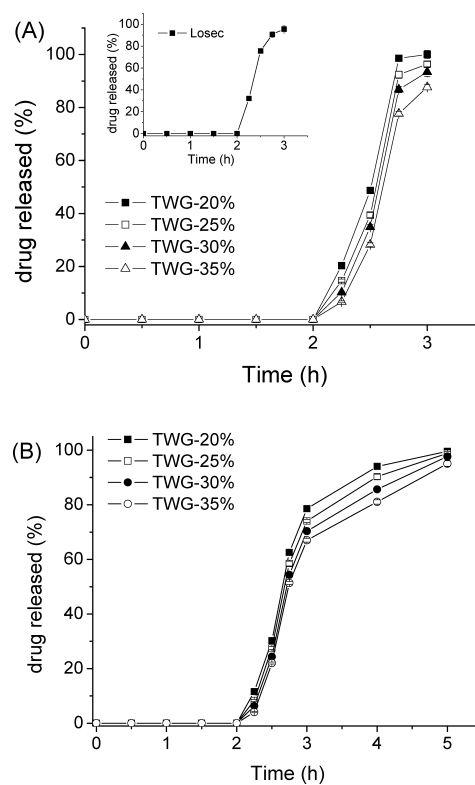


Fig. 5. Effect of Coating Level (%TWG) of Enteric Film Coating Layer on Drug Release from Two-Layer Coated Pellets (the Coating Levels for Salt and HPMC Layer Were TWG=35%, Respectively) after 2 h in 0.1 M HCl and 1 h in pH 7.4 Phosphate Buffer

(A) Drug-layered pellet cores, (B) drug-containing pellet cores.

results in Figs. 5A, B show the release of omeprazole from enteric coated pellets that were first subjected to 2 h in 0.1 M HCl followed by transfer into pH 7.4 phosphate buffers. No significant differences in enteric properties were established on increasing the coating level. As the thickness of the coating layer increased from 20 to 35, the lag time, *i.e.*, the delay in the period of time took to begin releasing omeprazole from the core, did not significantly increased. One of the most important properties of a modified release item is its resistance against gastric conditions. It is required that no more than 10% drug degradation would occur after 2 h in 0.1 M HCl solution. All formulations complied with the condition, and visual observation of the coated pellets with TWG=30 and 35% after 2 h in each acid yielded no signs of omeprazole degradation, which typically manifests itself by yellow or purple discoloration of the pellets, film layer, or dissolution media. However, when the enteric coating level was TWG=20 and 25%, slightly purple discoloration of the pellets in the film layer was observed from the multi-layer coated pellets after 2 h in 0.1 M HCl solution. The specifications were not less than 80% omeprazole dissolved after 45 min in pH 7.4 phosphate buffer. For the drug-layered pellets with multi-layer film coatings, drug release for each coating level fulfilled the criteria outlined in this study, *i.e.* not less than 80% dissolved after 45 min in buffer, pH 7.4. In comparison, multi-layer coated drug-containing pellets released no more than 65% omeprazole within 45 min. In other words, the multi-layer coated drug-layered pellets provided delayed and more rapid release of omeprazole than that from

drug-containing pellets. On the other hand, for the multi-layer coated drug-layered pellets, enhanced surface contact between the drug and the polymer increases the possibility for drug migration into the enteric film as well as the potential for drug-polymer interaction; Furthermore, omeprazole is a weak base, and enteric polymers are polymeric acids with free carboxyl groups. The drug would be induced to migrate into the enteric film. Thus, the subcoat (salt and HPMC layers) should be thick enough to prevent the drug migrating into the enteric layer. The rate of drug release from drug-layered pellets was slower than commercial Losec[®] within 30 min ($p > 0.05$); however, the drug release was also complete after 45 min. The different profiles of drug release might be attributed to the different formulations of pellets cores. In order to enhance *in vivo* gastric-resistance, the multi-layer coated pellets, whose the coating levels for salt, HPMC and enteric layer was TWG=35%, respectively, were selected for *in vivo* evaluation in rats and dogs.

pH-sensitive enteric films consist of a linear polymer chain with ionizable carboxyl groups. The methacrylic acid content in Eudragit[®] L-100 is between 46% and 50% and the polymer dissolves at a pH above 6. The pK_a and pH value of the polymethacrylates Eudragit[®] L-100 (Aqueous polymer dispersion 6%) were about 6.4 and 3.1, respectively. The acidic polymethacrylates Eudragit[®] L-100 showed a pronounced influence on the decomposition of omeprazole.⁹⁻¹¹ Given the tendency of enteric release polymers to degrade omeprazole, a subcoating is required. In present study, the salt and HPMC layers separated the omeprazole loading core from the enteric coating polymer(s) containing free carboxyl groups. Additionally, in order to further prevent omeprazole from degraded by enteric polymers, the pH value of aqueous dispersion of Eudragit[®] L-100 was adjusted to 5.0 before the coating process. At the same time, titanium pigment (TiO_2) was also added to the aqueous polymer dispersion which could enhance the stability of omeprazole in UV. During dissolution testing, the enteric-coating would swell, and dissolution medium could permeate into the film coating. Permeation of the dissolution medium into the pellet core would dissolve the omeprazole and sodium hydroxide, increasing the pellet core micro-environmental pH at the pellet core/film coating interface. When the pellet core micro-environmental pH exceeds pH 5.5, the enteric polymer will ionize and dissolve prematurely resulting in a more permeable membrane.

Stability Studies Figure 6 shows the remaining omeprazole vs. time relative to the initial assay. After the first, second, third, fourth, fifth and the sixth months, no visual discoloration of multi-layer coated pellets surface was observed. Samples stored for 6 months showed less than 6% loss of omeprazole. Specifically, after 6 months, the drug-layered and drug-containing pellets with multi-layer film coatings had omeprazole concentrations that averaged 94.0% and 95.2% of the initial concentrations, respectively.

After the first, second, third, fourth, fifth and the sixth months, the dissolution study was conducted in simulated gastro and small intestinal fluids as described above, no significant difference ($p > 0.05$) was observed in the cumulative percent of omeprazole released from the multi-layer coated pellets when compared to that released from the same formulation before storage (Fig. 7). The results indicate that storage at 40 °C/75% RH for 6 months have little influence on

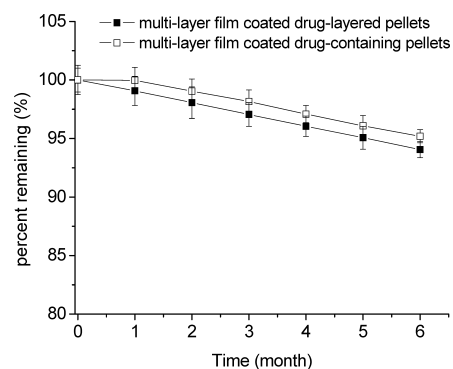


Fig. 6. Remaining Omeprazole (%) in Multi-layer Film Coated Pellets after Stored at 40 °C/75% Relative Humidity (RH) for 1, 2, 3, 4, 5 and 6 Months

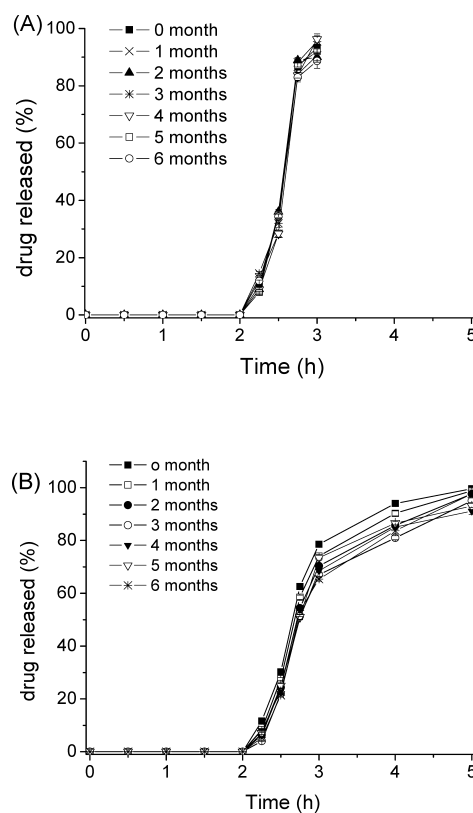


Fig. 7. *In Vitro* Dissolution Profiles of Multi-layer Film Coated Pellets after Stored at 40 °C/75% Relative Humidity (RH) for 1, 2, 3, 4, 5 and 6 Months

(A) Multi-layer film coated drug-layered pellets, (B) multi-layer film coated drug-containing pellets.

drug release. The insignificant changes in the physical appearance, drug content, and dissolution profile of the multi-layer coated pellets after storage at 40 °C/75% RH for 6 months indicate that the formulations could have a minimum shelf life of 2 years.²¹

***In Vivo* Gastro-Resistance and Stability in Upper GI Tract of Multi-layer Film Coated Pellets** After 0.25, 0.5, 1.0, 1.5 and 2.0 h, the multi-layer coated pellets were obtained from the gastro and small intestine in rats. The surface of coated pellets did not show any discoloration. No visual discoloration was observed from the ruptured pellets obtained from the small intestine.

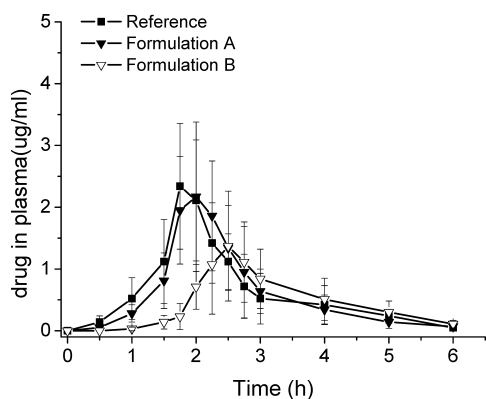


Fig. 8. Mean Plasma Omeprazole Concentration vs. Time Profile after Oral Administration of Multi-layer Coated Drug-Layered Pellets Capsules (Formulation A), Multi-layer coated Drug-Containing Pellets Capsules (Formulation B), or Losec® (Reference, Marketed Product) at a Dose of 3 mg/kg of Omeprazole Equivalent in Dogs

Omeprazole was stable enough at the conditions of pH 7.0 or higher.²²⁾ The pH value of small intestine was about 7.0. Thus, though omeprazole around the ruptured pellets was exposed to out conditions, it was still stable. Additionally, alkaline materials (NaOH and MgO) in the cores and film coating layers could create a “micro-pH” around omeprazole of not less than pH=7, preferably not less than pH=8, when water was adsorbed onto the cores. Hence, the alkaline materials could also enhance the stability of omeprazole in the gastro and absorption conditions (small intestine), which could improve the bioavailability of the multi-layer coated pellets.

Absorption Study in Dogs After oral administration of multi-layer coated drug-layered (Formulation A), drug-containing pellets capsules (Formulation B) and reference (Losec®) to dogs, the mean plasma concentrations–time profiles of omeprazole are shown in Fig. 8, and some relevant pharmacokinetic parameters are listed in Table 1. Though the C_{max} and T_{max} of Formulation A and Formulation B were not statistically significant ($p>0.05$), Formulation A showed a significantly higher C_{max} compared to Formulation B. The area under the plasma omeprazole concentration vs. time curves ($AUC_{0-\infty}$) for Formulation A and Formulation B were 3.48 ± 0.86 and $2.97\pm 0.57 \mu\text{g}\cdot\text{h}/\text{ml}$, respectively (Table 1), which were not significantly different from that obtained from the reference ($3.53\pm 0.87 \mu\text{g}\cdot\text{h}/\text{ml}$). Although there is little evidence of a correlation between C_{max} and the degree of acid suppression, a positive correlation between AUC and gastric pH is seen with omeprazole and lansoprazole.^{23,24)} Pharmacologic activity is dependent on the extent of omeprazole absorption. Therefore a similarity in the AUC is a very important factor for the evaluation of pharmacologic activity. Compared with Formulation B, Formulation A was more similar in the AUC to the reference. Based on the evidence of absorption in dogs, the AUC of Formulation A agrees with that of the reference. It was confirmed that the pharmacologic activity of Formulation A in dogs should be similar to that of the reference.

Oral delivery of poorly water-soluble drugs often results in low bioavailability since the rate-limiting step for absorption from the GI tract is a significantly slower dissolution rate. Furthermore, a poorly water-soluble drug required more time

Table 1. Omeprazole Pharmacokinetic Parameters after Oral Administration of Multi-layer Film-Coated Pellets or Losec® (Reference, Marketed Product) at a Dose of 3 mg/kg of Omeprazole Equivalent in Dogs

PK parameters	Reference	Formulation A	Formulation B
Ke (h^{-1})	0.82 ± 0.24	0.80 ± 0.42	0.53 ± 0.39
C_{max} ($\mu\text{g}/\text{ml}$)	2.34 ± 0.53	2.17 ± 0.17	1.39 ± 0.82
T_{max} (h)	1.75 ± 0.74	2.00 ± 0.15	2.51 ± 0.61
$T_{1/2}$ (h)	0.85 ± 0.41	0.87 ± 0.21	1.35 ± 0.51
AUC_{0-6} ($\mu\text{g}\cdot\text{h}/\text{ml}$)	3.48 ± 0.39	3.39 ± 0.72	2.61 ± 0.35
$AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{h}/\text{ml}$)	3.53 ± 0.87	3.48 ± 0.86	2.97 ± 0.57
$AUMC_{0-\infty}$ ($\mu\text{g}\cdot\text{h}^2/\text{ml}$)	9.19 ± 1.02	9.08 ± 0.48	9.99 ± 0.98
MRT (h)	2.60 ± 0.64	2.49 ± 0.32	3.33 ± 0.74
BA (%)		99.71 ± 4.65	84.12 ± 5.87

Ke : elimination rate constant, C_{max} : maximal plasma concentration, T_{max} : time to reach maximal plasma concentration, $T_{1/2}$: elimination half life, MRT : mean residence time, AUC : area under plasma concentration vs. time curve, $AUMC$: first moment curve, BA : relative bioavailability. Formulation A: multi-layer coated drug-layered pellets. Formulation B: multi-layer coated drug-containing pellets.

to dissolve in the GI fluid than it took to be absorbed in the GI tract.²⁵⁾ Omeprazole, a poorly water-soluble drug, was well absorbed from small intestine since the lipophilic drugs could permeate the intestinal membrane rapidly through the villous tips,²⁶⁾ and the rate of drug release can influence the total extent of absorption of omeprazole to the general circulation, and releasing the active drug rapidly from a pharmaceutical dosage form in the proximal part of the gastrointestinal canal would result in a good bioavailability.²⁶⁾ Thus, rapid release of omeprazole from the multi-layer coated drug-layered pellets could increase the bioavailability, and was higher than that obtained from multi-layer coated drug-containing pellets. On the other hand, when a great quantity of drug transit through the stomach to the small intestine, because a considerable amount of drug was released at the same time, the first pass metabolism for omeprazole was saturable in liver within a short time,^{27,28)} which would also increase the bioavailability. In addition, many factors affect the BA of omeprazole. They are: (1) degradation in the acid, (2) dissolution rate in the small intestine, (3) membrane permeability and (4) metabolism in the liver.²⁹⁾ Omeprazole belongs to the class II compound with low solubility and high membrane permeability according to the classification by Amidon *et al.*^{30,31)} Therefore, the solubility problem is the priority number one project to solve. As compared to the stomach, there is less water in the small intestine. Therefore, the dissolution rate of omeprazole from the multi-layer coated pellets has an important role on the bioavailability of omeprazole. To increase the dissolution rate of omeprazole, the drug was sprayed and layered on the pellet cores, which existed in an amorphous form, and surfactant was added in the drug layer. Thus, the bioavailability of omeprazole from the drug-layered with multi-layer film coatings was higher than that from drug-containing pellets.

Conclusions

The multi-layer coated pellets for omeprazole were developed. The system consists of drug-layer core pellets, coated with a salt layer, a HPMC layer and an enteric layer, respectively. The system could significantly improve the dissolution rate and stability of omeprazole. Salt layer improved the drug stability, and its coating levels had little influence on the dissolution profiles of omeprazole. The rate of drug release was

significantly delayed by HPMC coating levels. The salt layer could function as a separated layer, and substitute part of the HPMC layer and decrease the coating levels of HPMC. The multi-layer coated pellets provided delayed and rapid release of omeprazole, and the dissolution rate from multi-layer coated drug-layered pellets was much faster than that obtained from drug-containing pellets. The multi-layer coated pellets storage at 40 °C/75% RH for 6 months had no effect on the drug release, and no significant changes in the physical appearance, drug content were established. In addition, the multi-layer coated pellets were stable in the *in vitro/in vivo* gastric pH conditions. Rapid release of drug in small intestine could increase the *AUC* of omeprazole, the extent of absorption from the multi-layer coated drug-layered pellets was better than that obtained from drug-containing pellets; the similar *AUC* between multi-layer coated drug-layered pellets capsules and Losec[®] implies a similar inhibiting effect of the two pharmaceutical preparations on gastric acid secretion.

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