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Research paper

In vitro and *in vivo* evaluation of tegaserod maleate pH-dependent tablets

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Abstract

The purpose of this study was to prepare tegaserod maleate (TM) pH-dependent tablets and evaluate their advantages as a sustained release delivery system. TM, insoluble in water and unstable in gastric milieu, was formulated into pH-dependent tablets coated with combinations of two methacrylic acid copolymers – Eudragit® L100 and Eudragit® S100. The influence of core tablet compositions, polymer combination ratios and coating levels on the *in vitro* release rate of TM from coated tablets was investigated. The optimum formulation was evaluated for *in vitro* release rate and *in vivo* bioavailability study on beagle dogs. In addition, physico-chemical properties of the drug, including solubility at different pH and temperatures, and dissociation constant were determined. The results showed that no drug was released in 0.1 mol/L hydrochloric acid within 2 h, and about 90% of the drug was released in the pH 6.8 phosphate buffer within 12 h in a sustained manner. The pharmacokinetic investigation showed that TM pH-dependent tablets exhibited a sustained plasma concentration, a lag time of approximately 2.3 h and a relative bioavailability of 159% compared to plain tablets. A close correlation existed between the *in vitro* release rate of the pH-dependent system and its *in vivo* absorption percentage. The results of the present study have demonstrated that the pH-dependent tablet system is a promising vehicle for preventing rapid hydrolysis in gastric milieu and improving oral bioavailability of TM for the treatment of irritable bowel syndrome.

Keywords: Tegaserod maleate; pH-dependent; Bioavailability

1. Introduction

Tegaserod maleate (TM), 3-(5-methoxy-1H-indol-3-ylmethylene)-*N*-pentyl-carbazimidamide hydrogen maleate (Fig. 1), is the first selective 5-hydroxytryptamine type-4 (5-HT_4) receptor partial agonist used for the treatment of constipation predominant irritable bowel syndrome (IBS), a complex gastrointestinal disorder characterized by a combination of abdominal pain, discomfort, diarrhea, and/or constipation [1]. IBS is highly prevalent in the general population and is associated with significant disability and health care costs. Its prevalence is known to be underreported and may range from 2.9% to 20.0% in the United States [2].

TM is insoluble in water and has a pH-dependent solubility, and its solubility is about 10-fold lower at pH 7.5 than at pH 1. Below pH 3, TM is rapidly degraded through hydrolytic breakdown [2]. TM is rapidly absorbed following oral administration under fasted conditions, and the peak plasma concentration (C_{max}) occurs after 1.0–1.3 h. Absolute bioavailability is about 10%, and terminal elimi-

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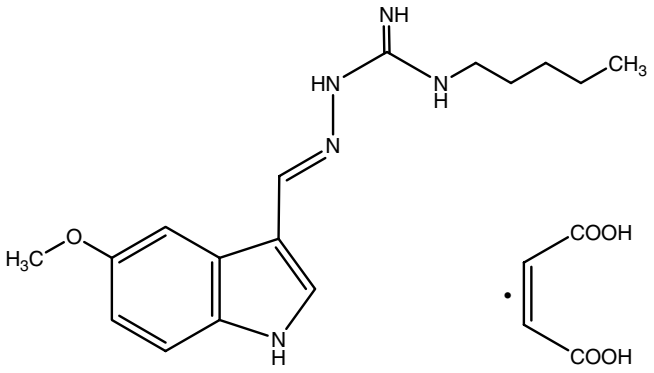

Fig. 1. Chemical structure of tegaserod maleate.

nation half-life is close to 11 h. Food reduces the area under the curve (AUC) by approximately 50% and C_{max} by 20–40%. Approximately two-thirds of the drug administered is excreted unchanged in the feces, with the remainder eliminated in the urine as inactive metabolites. In the stomach, TM is hydrolyzed by the acid, followed by hepatic oxidation and conjugation to the major inactive metabolite, 5-methoxy-indole-3-carboxylic acid glucuronide. TM also undergoes direct glucuronidation to 3 isomeric *N*-glucuronides. About 98% of the drug is bound to plasma proteins, predominantly with α_1-glycoprotein. The pharmacokinetics of TM is not affected by gender [2,3].

To improve the oral bioavailability and prevent rapid hydrolysis of TM in gastric milieu, a dosage form containing TM in a core is coated with pH-dependent materials which dissolve at the pH of the small intestine. The pH values of the stomach and the small intestine in humans are 1–3 and 6.5–7, respectively [4]. The most commonly used pH-dependent coating polymers are methacrylic acid copolymers – Eudragit® L100 and Eudragit® S100 which dissolve at pH 6.0 and 7.0, respectively. Hence, neither polymer is suitable to be used alone for coating dosage forms that start releasing the drug at pH 6.5. Since Eudragit® S100 dissolves at the higher pH within the range, it would be possible to combine Eudragit® S100 with Eudragit® L100 at various ratios to manipulate the drug release within the pH range of 6.0–7.0. To date, no report is available regarding the use of a combination of pH-dependent polymers to deliver TM for treating IBS. To our knowledge, most of the literature on tegaserod focused on the pharmacology and human pharmacokinetics with no emphasis on formulation evaluation and animal pharmacokinetics [5–12].

The objective of this study was to develop a pH-dependent tablet formulation of TM that would allow the dosage form to pass through the stomach intact, start disintegrating at the upper small intestine and slowly release the drug. In addition to the optimization of the *in vitro* drug release profiles by evaluating the influence of formulation and coating parameters, pharmacokinetic investigation on beagle dogs was also performed to evaluate the coated tablets.

2. Materials and methods

2.1. Materials

TM was synthesized by the Department of Organic Chemistry, Shenyang Pharmaceutical University (China). Diphenhydramine hydrochloride (Shenyang Pharmaceutical Inc., Shenyang, China) as an internal standard, lactose (Shenyang Xinxi Reagent Co., Shenyang, China) as a diluent, poloxamer 188 (Shenyang Pharmaceutical Inc., Shenyang, China) as a solubilizer, sodium carboxymethylstarch (CMS-Na) (Shanghai Yongri Co. Ltd., Shanghai, China) as a disintegrant povidone K30 (Tianjin Tiantai Fine Chemical Co., Tianjin, China) as a binder, diethyl phthalate (DEP) (Tianjin Reagent Co., China) as a plasticizer and polyethylene glycol 400 (PEG 400) (Shenyang Chemicals Co., Shenyang, China) as a pore former were obtained from the indicated sources. Methacrylic acid copolymers (Eudragit® L100 and Eudragit® S100) were kindly donated by Degussa-Röhm Co. (Darmstadt, Germany). Acacia, sodium dodecylsulfate (SDS), low-substituted hydroxypropyl cellulose (L-HPC) and sodium cross-linked carboxymethyl cellulose (CCNa) were provided by Shanghai Yongri Co. Ltd (Shanghai, China).

2.2. HPLC analysis of tegaserod

The quantitative determination of TM was performed using the Shimadzu HPLC system equipped with a SPD-10A VP detector (Shimadzu Inc., Kyoto, Japan) at 220 nm. A mobile phase consisting of acetonitrile−0.003 mol/L phosphate buffer (40: 60, v/v) was pumped through a Kromasil C_8 column (200 mm × 4.6 mm, 5 μm; Turner, Tianjin, China) at a flow rate of 1.0 ml/min. A run time of 10 min at ambient temperature was used. The retention time of TM was 7.0 min.

TM degraded rapidly into 5-methoxy-indole-3-formaldehyde (MIF) below pH 3 [2]. The release of TM from the coated tablets in 0.1 mol/L hydrochloric acid (HCl) was determined by incubating the dissolution samples in a water bath at 80 °C for 10 min. The concentration of MIF was measured by HPLC to calculate the amount of the drug released. The retention time of MIF was 4.7 min.

2.3. Preformulation studies

2.3.1. Solubility

An excess amount of TM was weighed into conical flasks to which 10 ml of pH 5.01, 5.96, 6.83 7.70, 9.96 and 12.00 buffer solutions with ionic strength of 0.276 was added, respectively. The samples were sonicated for 10 min at room temperature, and the capped conical flasks were shaken for 48 h at 25 ± 0.1 °C. Samples were collected, filtered through 0.45-μm membrane filters, diluted appropriately and analyzed spectrophotometrically at 310 nm. The Gibbs energy of the dissolution processes ΔG°_{sol} was calculated as follows:

$$\Delta G^{\circ}_{sol} = -RT \ln X_2$$

where X_2 is the drug molar fraction in the saturated solution.

Furthermore, the solubility of TM in water at 5, 20, 37 and 50 °C was also determined. The mole dissolution enthalpy, $\Delta_s H$, of TM was calculated using the following equation:

$$\ln X_2 = -\frac{\Delta_s H}{R} \cdot \frac{1}{T} + C$$

where C is the intercept.

2.3.2. Dissociation constant

The dissociation constant (K_a) of TM was determined by a spectrophotometric method [13]. pK_a was calculated by the following equation:

$$pK_a = pH + \lg \frac{A_M - A}{A - A_I}$$

where A_M is absorbance of the fully unionized form of the drug, A_I is absorbance of the fully ionized form of the drug and A is absorbance at the given pH.

At 20 °C, the values of A_I and A_M were determined in the acidic environment (pH 4.02 and 6.22) and basic environment (pH 12.20 and 12.80) at 310 nm, where the ionized and unionized forms of the drug had appreciably different extinction coefficients. The values of A were measured at pH 9.10, 9.40, 9.60, 10.00 and 10.22 of buffer solutions. The ionic strength of the buffer solutions used was adjusted to 0.16 with sodium chloride.

2.4. Preparation of core tablets

TM core tablets were manufactured by a wet granulation method. TM, lactose, CMS-Na and poloxamer 188 were tumble-mixed, and 10% w/v of povidone K30 in ethanol was mixed with the powder blend, and the wet mass was passed through an 18-mesh standard sieve. The wet granules were dried in the air dryer at 44 °C for 1 h, screened through the 16-mesh sieve, and then compressed into tablets with a hardness of 38 N using a single-punch tableting machine (Model TDP, Shanghai First Pharmaceutical Machinery Factory, Shanghai, China) fitted with a 6-mm diameter shallow biconcave punch and die set. The resulting tablets, weighing 100 mg each, contained approximately 8.3 mg of TM each (equivalent to 6.0 mg of tegaserod).

2.5. Coating of core tablets

A Eudragit® L100 and Eudragit® S100 combination solution in ethanol (5%, w/w) was prepared, and DEP (1%, w/w) as well as PEG 400 (1%, w/w) were slowly stirred into the polymer solution. The coating of TM tablets was performed in a mini-film-coating unit (Model BY300A, Taixing Second Pharmaceutical Machinery Factory, Taixing, China). After the core tablets were warmed at 30 °C for 10 min, the coating solution was sprayed through a 0.75-mm spray nozzle at a flow rate of 5 ml/min, the pneumatic spraying pressure being 0.6 MPa. The inlet and outlet temperatures of the drying air were 35 and 30 °C, respectively. The pan rotation speed was set at 25 rpm. After being coated to a 5.0% coating level (weight gain), the tablets were dried at 40 °C for 6 h to remove the residual solvent, and stored in a desiccator until analysis.

2.6. Optimization of formulation parameters

To optimize the release rate of TM from the coated tablets, the influence of the following factors was investigated. The composition of tested core tablet formulations is presented in Table 1.

(1) Solubilizer: Acacia and SDS were studied at a level of 20% (w/w), while the influence of poloxamer 188 was investigated at 10%, 20% and 30% weight ratios.
(2) Disintegrant: L-HPC and CCNa were studied at a level of 20% (w/w), while the influence of CMS-Na was investigated at 10%, 20% and 30% weight ratios.
(3) Ratio of Eudragit® L100–Eudragit® S100: Eudragit® L100–Eudragit® S100 combination ratios of 1:0, 3:1, 1:1, 1:2, 1:4 and 0:1 at the constant total amount of Eudragit® polymer (5%, w/w) were investigated.
(4) Coating level: In addition to a 5% coating level, 1.5% and 7.3% levels were investigated at the Eudragit® L100–Eudragit® S100 combination ratio of 1:4.

2.7. In vitro drug release study

Release studies were conducted using a USP method II paddle dissolution apparatus (Tianjin University Radio Inc., Tianjin, China) at a rotation speed of 50 rpm at 37 ± 0.5 °C. The dissolution media used were 900 ml of 0.1 mol/L HCl for 2 h, followed by 900 ml of pH 6.8 phosphate buffer solution for 12 h. Samples (5 ml) withdrawn at predetermined time intervals were passed through 0.45-μm

Table 1
Composition of core tablets loaded with 8.3% (w/w) tegaserod maleate

Composition (%)	Formulation								
	A	B	C	D	E	F	G	H	I
Lactose	50	50	50	50	50	60	40	60	40
Solubilizer									
Acacia	20	–	–	–	–	–	–	–	–
SDS	–	20	–	–	–	–	–	–	–
Poloxamer 188			20	20	20	10	30	20	20
Disintegrant									
CMS-Na	20	20	20	–	–	20	20	10	30
L-HPC	–	–	–	20	–	–	–	–	–
CCNa	–	–	–	–	20	–	–	–	–
Povidone K30	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

q.s. (quantum sufficit), as much as sufficient quantity; "–", not applicable.

membrane filters, and the amount of the drug released was measured at 310 nm by spectrophotometry.

To study the effect of the pH of the phosphate buffer media on the release of TM from optimum formulation, i.e., formulation C coated with Eudragit® Ll00–Eudragit® S100 combination ratio of 1:4 at the coating level of 5%, pH 6.2 and pH 7.4 Clark-Lubs phosphate buffer solutions were employed in the drug release study to account for the gastrointestinal pH variability among individuals.

2.8. Statistical analysis

As recommended by the FDA, the similarity factor f_2 was used as a determination for assessing the similarity of dissolution profiles [14,15]. The compared dissolution profiles were obtained under the same test conditions and their dissolution time points were the same. Two profiles were thought to be statistically similar if the f_2 value was greater than 50.

2.9. Pharmacokinetic study

The *in vivo* evaluation was performed by a crossover treatment in male beagle dogs with a washout period of 7 days. The beagle dogs were deprived of food overnight for 12 h, although they were allowed free access to drinking water. During the course of the experiment, water was not provided to the dogs until 6 h after the administration of two preparations. The two preparations studied included TM coated tablets (CT) and TM plain tablets (PT) containing 8.3 mg of TM each. Both treatments contained 8.3×4 mg of TM. All studies were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication #85-23, revised in 1985), and approved by the Department of Laboratory Animal Research at Shenyang Pharmaceutical University. Blood samples were collected at the following time-points: (1) 0 (pre-dosing), 2, 3, 4, 5, 6, 8, 10, 12, 14, 16 and 24 h for CT; (2) 0 (pre-dosing), 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16 and 24 h for PT. After centrifugation at 4000 rpm for 10 min, plasma samples were stored at −20 °C until analysis.

2.10. LC-MS/MS analysis of tegaserod in plasma samples

A Finnigan TSQ™ tandem mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source (Thermo Fisher Scientific Inc., MA, USA) was used for LC-MS/MS analysis. Chromatographic analyses were performed using a Diamonsil C_{18} column (250 mm × 4.6 mm, particle size 5 μm; Dikma Inc., Beijing, China) at ambient temperature. The mobile phase consisted of methanol–water–formic acid (80:20:1, v/v), delivered at a flow rate of 0.6 ml/min with a run time of 4 min. Quantification was performed using selected reaction monitoring (SRM) of the mass transitions of m/z 302 → 173 for TM and 256 → 167 for diphenhydramine hydrochloride, respectively, in the positive ion detection mode. The retention time of diphenhydramine hydrochloride and TM was 3.27 and 3.50 min, respectively.

An aliquot (0.5 ml) of plasma sample was measured into a glass tube with a Teflon-lined cap, followed by the addition of 0.1 ml of 120 ng/ml diphenhydramine hydrochloride solution, 0.5 ml of water, 0.1 ml of 1 mol/L sodium hydroxide and 3 ml of ethyl ether. The mixture was vortexed for 5 min and centrifuged at 4000 rpm for 10 min. The ethyl ether layer was transferred to another glass tube and dried under a stream of nitrogen gas at 40 °C. The residue was reconstituted with 0.1 ml of mobile phase, vortexed for 5 min, and 20 μl aliquot of each sample was injected into LC-MS/MS.

2.11. Pharmacokinetic data analysis

The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were recorded as observed. Half-life ($T_{1/2}$), lag time (T_{lag}) and mean residence time (MTR) were calculated using the 3P87 software, a practical pharmacokinetic program (the Chinese Society of Mathematical Pharmacology, Beijing, China). The area under the plasma concentration–time curve (AUC) was calculated using the trapezoidal rule. Initially AUC_{0-24h} was calculated using the trapezoidal rule, and $AUC_{0-\infty}$ was obtained using the following equation:

$$AUC_{0-\infty} = AUC_{0-24h} + C/\lambda_z$$

where C is the plasma concentration of TM at 24 h, and λ_z is the terminal elimination rate constant. The value of λ_z was calculated by subjecting the last three points of the semilogarithmic plot of time versus drug plasma concentration to regression analysis. The relative bioavailability of TM from CT when compared with PT was calculated by dividing its $AUC_{0-\infty}$ with that of PT. All values were expressed as means ± standard deviation (SD). The comparison for the difference between parameters was analyzed using a one-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Solubility and dissociation constant

The solubility of TM in various pH buffer solutions and at different temperatures is shown in Fig. 2 and Table 2, respectively. The solubility of the drug in increasing pH buffer solutions showed a V-shaped curve, and the minimum and maximum solubility were found at pH 7.70 (22.7 μg/ml) and pH 12.00 (102.4 μg/ml), respectively. ΔG°_{sol} values were positive, ranging from 30.6 to 34.3 kJ/mol at the pH interval of 5.01–12.00, indicating the non-spontaneous nature of the drug dissolution. The solubility of the drug at 20 and 37 °C was found to be 93.9 and 210.8 μg/ml, respectively. Therefore, according to the USP solubility definition, TM can be considered a very

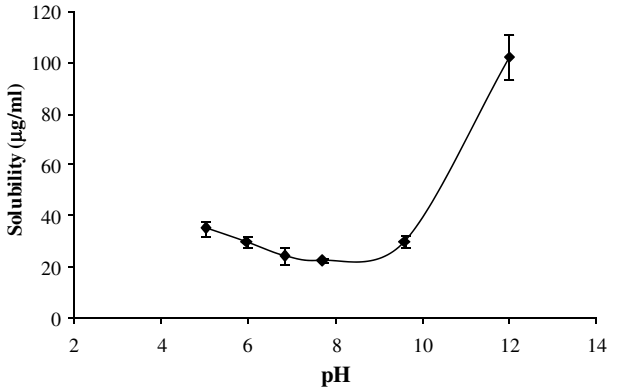

Fig. 2. The solubility of tegaserod maleate in various pH buffer solutions at 25 °C.

Table 2
The solubility of tegaserod maleate at different temperatures

Temperature (°C)	5	20	37	50
Solubility (μg/ml)	38.4 ± 2.5	93.9 ± 6.2	210.8 ± 14.8	425.1 ± 24.3

Data are presented as means ± SD.

slightly soluble drug at 37 °C. The value of $\Delta_s H$ was 39.3 kJ/mol calculated from the linear regression curve with a correlation coefficient of 0.999, demonstrating that the dissolution of the drug was an endothermic process and the solubility increased with elevated temperature.

The value of pK_a was found to be 9.76 ± 0.03. To measure a reliable pK_a value of a weak base, the pH range used should cover solutions where the lowest pH ensures practically full protonation of the base, and the highest pH brings no protonation, i.e., neutral species. By using the well-known Henderson–Hasselbach equation, one obtains the pH range ± 3 pH units around the expected pK_a. In this case, the expected pK_a was approximately 9.5; and therefore, the pH range used should cover pH 6–13. Buffer capacity was considered in the determination of pK_a. Too low of a buffer capacity would result in insufficient pH control in the analyte zone, which would lead to a bias in the final pK_a determination. Hence, the buffers should be selected in such a way that the operational pH is within pK_a ± 1, where pK_a corresponds to the buffering species. In this study, five buffer solutions with pH ranges of 9.1–10.2 were selected.

3.2. In vitro drug release

3.2.1. Effects of solubilizer and disintegrant

Drug release profiles of formulations A to I using a Eudragit® L100–Eudragit® S100 ratio of 1:4 at the coating level of 5% are presented in Fig. 3. Formulations A and B employing acacia and SDS as solubilizers, respectively, showed a significantly longer lag time (5 h) as compared to formulation C containing poloxamer 188 (2 h), and the similarity factor f_2 was 21.0 and 26.7 for formulations A and B, respectively. These results may be explained since

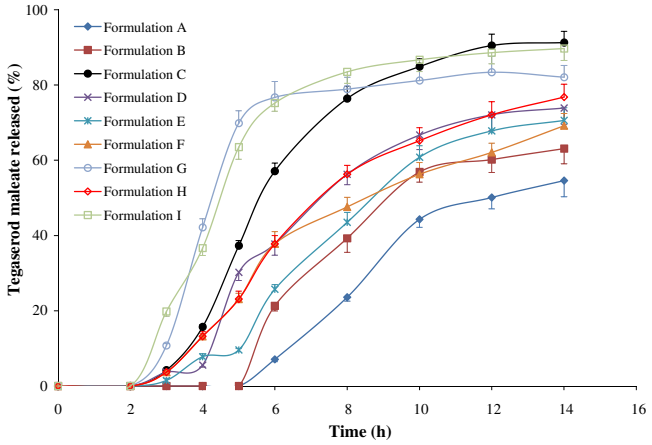

Fig. 3. Drug release profiles of tegaserod maleate tablets with various solubilizers and disintegrants using the Eudragit® L100–Eudragit® S100 combination ratio of 1:4 at the coating level of 5% in 0.1 mol/L hydrochloric acid for 2 h, followed by the pH 6.8 phosphate buffer solution for 12 h at 37 °C ($n = 6$).

anionic surfactants, such as acacia and SDS, are prone to forming a complex with a cationic drug which hinders further dissolution of the drug. In addition, the cations in the pH 6.8 phosphate buffer solution coagulate with acacia and SDS and reduce their solubilization effects. The formulations D and E, employing L-HPC and CCNa as disintegrants, respectively, exhibited significantly lower drug release than formulation C containing CMS-Na, and f_2 was 40.8 and 31.6 for formulations D and E, respectively. The drug release rate and extent increased with increasing concentrations of poloxamer (formulation G > formulation C > formulation F) and CMS-Na (formulation I > formulation C > formulation H). Therefore, 20% poloxamer and 20% CMS-Na were selected as the solubilizer and the disintegrant, respectively, in the core tablets for the optimum formulation.

3.2.2. Effects of the ratio of Eudragit® L100 to Eudragit® S100 and coating level

The release profiles of tablets coated with various ratios of polymers and different coating levels are shown in Figs. 4 and 5, respectively. All of the coated formulations did not release any TM in 0.1 mol/L HCl within 2 h, indicating that the pH-dependent tablet formulation would be intact during its transit through the stomach. The tablets coated with Eudragit® L100 only (Formulation 1:0) demonstrated a fast release (about 67% of the drug) within 1 h in the pH 6.8 buffer media. The release rates decreased significantly with the introduction of Eudragit® S100 in the formulations. As expected, tablets coated with only Eudragit® S100 (formulation 0:1) exhibited the slowest release rate, and started releasing TM after 3 h and released approximately 45% of drug at the end of release study in the pH 6.8 buffer media. Combination formulations containing only 20–33% of Eudragit® L100, i.e., formulations 1:2 and 1:4, finally released about 90% of the drug. The drug

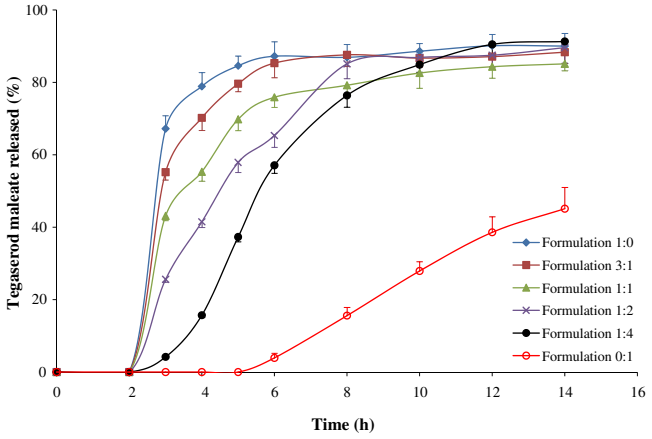

Fig. 4. Drug release profiles of tegaserod maleate tablets coated with the Eudragit® L100–Eudragit® S100 combination ratios of 1:0, 3:1, 1:1, 1:2, 1:4 and 0:1 at the coating level of 5% in 0.1 mol/L hydrochloric acid for 2 h prior to the pH 6.8 phosphate buffer solution for 12 h at 37 °C ($n = 6$).

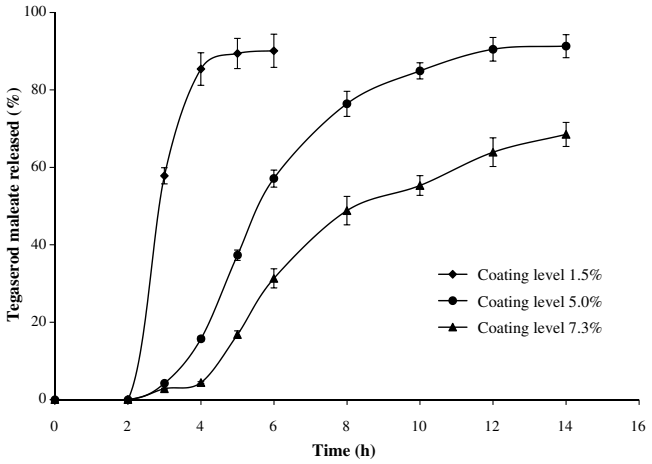

Fig. 5. Drug release profiles of tegaserod maleate tablets coated with the Eudragit® L100–Eudragit® S100 combination ratio of 1:4 at the coating level of 1.5%, 5.0% and 7.3% in 0.1 mol/L hydrochloric acid for 2 h prior to the pH 6.8 phosphate buffer solution for 12 h at 37 °C ($n = 6$).

release behaviors from coated tablets with different coating levels showed a typical sigmoidal pattern characterized by a distinctive lag time. The lag time extended with an increase in the coating level. Rapid release of TM was observed from the tablets with a coating level of 1.5% and a sustained release from those coated at a level of 7.3%. This could be attributed to a longer time taken to solubilize the thicker film at higher coating levels. Although the tablets coated at a level of 7.3% exhibited sustained release, only 60% of the drug was released at the end of 12 h in the pH 6.8 phosphate buffer media. On the contrary, tablets coated at a level of 5% showed 90% drug release at the end of 12 h and were therefore selected as the optimum formulation for further analysis (Fig. 5).

3.2.3. Effect of pH

Based on the above studies, the optimum formulation, i.e., formulation C coated with Eudragit® L100–Eudragit® S100 at a combination ratio of 1:4 and at the coating level of 5%, was chosen for studying the effect of pH of the buffer media on the release profiles, as shown in Fig. 6. As anticipated, the release profiles were obviously faster in pH 7.4 than in pH 6.2, and no significant difference in drug release behavior was observed between the pH 6.8 and pH 7.4 buffer media.

3.2.4. Release mechanism

The coated films from all of the combinations were soluble within the pH range of 6.0 to 7.0, at which pH Eudragit® L100 and Eudragit® S100 start dissolving, respectively. The increase in the Eudragit® L100 content of the film resulted in a shorter time for the polymer dissolving to release the drug. The solubility of the films from various combinations of Eudragit® L100–Eudragit® S100, and the release rate of drug from the coated tablets in various pH media could be controlled by varying the ratios of the two polymers. The release mechanism may be attributed to a change in permeability of the film caused by a salt formation of the carboxylic acid groups in Eudragit® L100 and Eudragit® S100 with anions in the buffer media, of which some of the film dissolves and releases from the core. The release media then penetrate into the core, resulting in disintegration of the tablets or loss of integrity of the film. The film permeability should increase with a higher amount of Eudragit® L100 in the film since Eudragit® L100 has a higher ratio of free carboxyl groups than Eudragit® S100. This would lead to a gradual increase in the drug release as the polymers dissolve. The dissolution of two polymers therefore is rate-limiting. The tablets with the higher coating levels showed increased release time compared to those with lower coating levels, demonstrating the effect of coating thickness on the release rate which was expected due to the longer time taken to solubilize the thicker film.

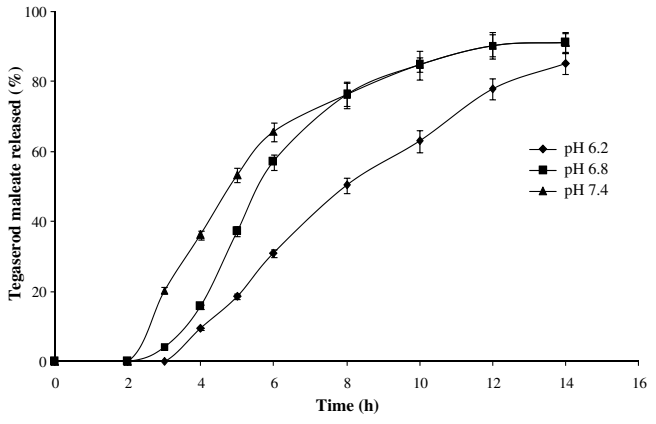

Fig. 6. Drug release profiles of tegaserod maleate tablets coated with the Eudragit® L100–Eudragit® S100 combination ratio of 1:4 at the coating level of 5.0% in 0.1 mol/L hydrochloric acid for 2 h followed by the pH 6.2, 6.8 or 7.4 phosphate buffer solutions for 12 h at 37 °C, respectively ($n = 6$).

3.3. Pharmacokinetic study

Analytical methods utilized were validated according to the FDA guidelines and requirements [16]. No endogenous or extraneous peaks were observed interfering with the separation and quantification of TM or diphenhydramine hydrochloride. A good linear correlation with coefficient greater than 0.996 was obtained between the ratio of peak area of TM to internal standard and the known TM concentration in the range of 1–200 ng/ml. The extraction recovery, accuracy and precision were evaluated at the levels of 2, 15 and 100 ng/ml using six determinations per concentration on different days. The accuracy was expressed as percentage value (% accuracy = [measured concentration/nominal concentration] × 100%). The precision was presented as percentage relative standard deviation (%RSD). The intra- and inter-day accuracy at all levels fell in the ranges of 100.72–102.75% and 100.61–105.45%, and the intra- and inter-day precision were in the ranges of 4.20–5.74% and 1.90–4.17%, respectively. The mean extraction recovery at all concentrations was 85.0–88.0%. The lower limit of quantification of TM was 0.2 ng/ml.

The mean plasma concentration–time profiles following oral administration of two kinds of TM preparations are shown in Fig. 7. The calculated pharmacokinetic parameters are summarized in Table 3. The results of PT indicated that TM could be rapidly absorbed from the dog gastrointestinal tract with a C_{max} of 33.38 ± 4.55 ng/ml at a T_{max} of 2.17 ± 0.76 h, and did not exhibit any lag time prior to drug release. On the other hand, a lag time of 2.33 ± 0.58 h was observed after oral administration of CT, and C_{max} and T_{max} were 18.07 ± 1.31 ng/ml and 8.00 ± 2.00 h, respectively.

The oral administration of CT resulted in a bioavailability of 304.13 ± 44.08 h ng/ml, whereas the oral administration of PT produced an $AUC_{0-\infty}$ of 190.82 ± 22.46 h ng/ml. The $AUC_{0-\infty}$ of CT was 1.59-fold higher than that of PT, denoting a statistically significant difference between the two values ($p < 0.05$). Also, the MRT of CT (11.61 ± 2.84 h) was found to be significantly higher ($p < 0.05$) than that of PT (6.17 ± 1.39 h). Compared to PT, CT showed a lower C_{max} and prolonged T_{max} in the plasma concentration–time profile, which was due to the barrier properties of the coated film of CT leading to drug release delay and rate-controlled release.

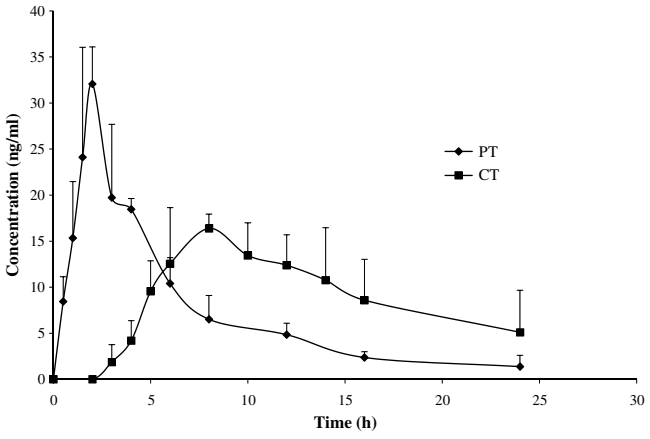

Fig. 7. Plasma concentration–time profiles of tegaserod maleate after oral administration of plain tablets (PT) and coated tablets (CT) to beagle dogs ($n = 3$).

Table 3
Pharmacokinetic parameters obtained after a single oral dose of tegaserod maleate coated tablets (CT) and plain tablets (PT) in dogs

Parameters	CT	PT
$AUC_{0-\infty}$ (h ng/ml)	304.13 ± 44.08[a]	190.82 ± 22.46
C_{max} (ng/ml)	18.07 ± 1.31[b]	33.38 ± 4.55
T_{max} (h)	8.00 ± 2.00[b]	2.17 ± 0.76
$T_{1/2}$ (h)	9.83 ± 6.27	6.36 ± 2.18
MRT (h)	11.61 ± 2.84[a]	6.17 ± 1.39
T_{lag} (h)	2.33 ± 0.58[b]	0.00 ± 0.00

Data are expressed as means ± SD ($n = 3$).
[a] $p < 0.05$, compared with PT.
[b] $p < 0.01$, compared with PT.

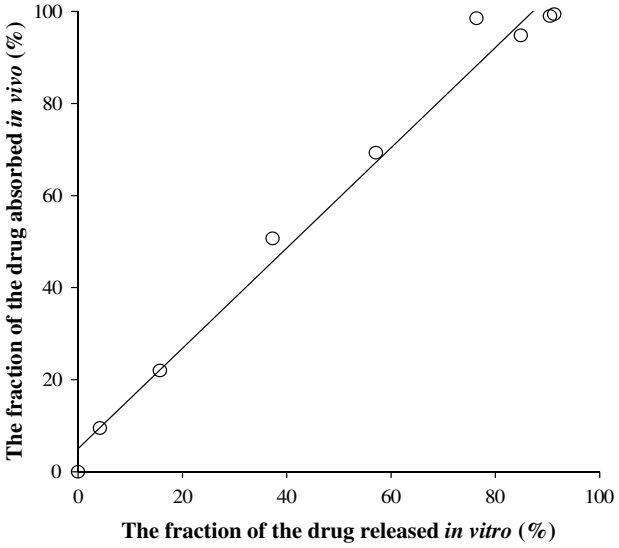

Fig. 8. The correlation of the fraction of the drug absorbed *in vivo* and the fraction of the drug released *in vitro* for tegaserod maleate coated tablets.

The correlation of the fraction of the drug absorbed *in vivo* and the fraction of the drug released *in vitro* for TM coated tablets is shown in Fig. 8. The *in vivo* absorption of TM exhibited a delay with a lag time of around 2.3 h, correlating well to the *in vitro* lag time of 2 h. The absorption was close to zero order with a slight decrease after 8 h. When the fraction of the drug absorbed *in vivo* was plotted versus the fraction of the drug released *in vitro*, a good linear correlation was obtained with a correlation coefficient of 0.992. These findings indicate that a close correlation can be established between the *in vitro* release rate of a coated system and its *in vivo* absorption percentage.

4. Conclusions

Various factors such as core tablet compositions, polymer combination ratios and coating levels influenced the release

of TM from pH-dependent tablets. The lag time prior to drug release was highly influenced by coating level. The release rates decreased significantly with an increase in concentration of Eudragit® S100 in the formulations. Based on the effects of the polymer combination ratios and coating level on the drug release, TM pH-dependent tablets coated with the Eudragit® L100–Eudragit® S100 combination ratio of 1:4 at a coating level of 5% were developed. The *in vitro* release study showed that none of the drug was released in 0.1 mol/L HCl within 2 h, and about 90% of the drug was released in the pH 6.8 phosphate buffer within 12 h in a sustained manner. The drug release mechanism involved a change in permeation of the film caused by salt formation in the coated film. The pharmacokinetic investigation on beagle dogs showed that TM pH-dependent tablets exhibited a sustained plasma concentration, a lag time of approximately 2.3 h and a relative bioavailability of 159% compared to plain tablets. A close correlation existed between the *in vitro* release rate of the pH-dependent system and its *in vivo* absorption percentage. The results of the present study have demonstrated that the pH-dependent tablet system is a promising vehicle for preventing rapid hydrolysis in gastric milieu and improving oral bioavailability of TM for the treatment of IBS.

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