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Research paper

Biphasic release of indomethacin from HPMC/pectin/calcium matrix tablet: II. Influencing variables, stability and pharmacokinetics in dogs

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Abstract

The pectin/calcium interaction, which is the basis for biphasic release of indomethacin from the HPMC/pectin/calcium chloride matrix tablet, is susceptible to influence of a variety of variables that is supposed to be encountered by the oral route. In this study, the effect of influencing variables on biphasic release characteristics, the stability and the pharmacokinetics of the hybrid matrix tablet were investigated. An increasing tendency of the overall release rate was observed from pH 1.2 to 7.4. The power law correlation n values increased with pH, while the release lag time or 10% release time ($T_{0.1}$) decreased at pH 6.8 and 7.4. Ionic strength in the release media also influenced the biphasic release significantly at sodium chloride levels of over 0.5%. Obvious increase in overall release rate was observed at sodium chloride level of 0.9% with an n value of 1.20 and a $T_{0.1}$ of 3.4 h. At sodium chloride levels of over 2%, the pectin/calcium interaction was disrupted resulting in very fast release of indomethacin. Release in gradient pH media was similar to that in pH 6.8 citrate buffer. When pectinase (Pectinex Ultra SP-L) was added into the release medium in 22.2 pg/ml or over, obvious triggering on drug release was observed. The stress testing showed increased release at extreme relative humidity of 92.5%. Both accelerated testing for 6 m and long-term testing for 12 m affirmed fine stability, especially in release characteristics. Pharmacokinetic study in dogs gave T_{max}/C_{max} of 4 h/604 ng/ml and 3 h/1662 ng/ml for HPMC/pectin/calcium and HPMC/pectin tablet, respectively. The plasma indomethacin level of the calcium-containing tablet was maintained at a much lower level for 3 h with a MRT of 7.13 h, longer than 3.97 and 5.61 h for indomethacin crude drug and HPMC/pectin tablet, confirming delayed absorption. The AUC of the HPMC/pectin/calcium tablet was lower than that of the HPMC/pectin tablet and indomethacin crude drug showing incomplete absorption. It is concluded that the HPMC/pectin/calcium matrix tablet is potentially useful for colon-specific drug delivery.

Keywords: Pectin; HPMC; Calcium; Matrix; Biphasic release; Pharmacokinetics

1. Introduction

Pectin is a naturally occurring polyanionic polymer that found potential use in the field of drug delivery [1–3]. To overcome its high hydrophilicity, calcium-induced crosslinking of pectin chains has been employed to achieve tight controlling on drug release, such as the systems based on calcium pectinate [4,5] and in situ crosslinking matrix comprised of pectin and calcium salt [6,7].

In previous studies with in situ crosslinking pectin/calcium chloride [7] and HPMC/pectin/calcium chloride matrix tablets [8], biphasic, slow initially and quick at later stages, release of a water-insoluble drug indomethacin has been investigated. As a result of calcium-induced crosslinking of pectin chains, significant retardation on drug release was observed. Interesting findings include: (1) suppression of drug release was observed up to calcium chloride, highly water-soluble substance that may perform as tunneling agent, level of about 60%; (2) characterization of the

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biphasic release by the power law equation gave exponent n values much higher than 1.0; (3) erosion was confirmed to be the underlying release mechanism. The biphasic release showed a characteristic initial release lag time, defined as 10% release time, which has been tuned by adjusting the formulation variables to as long as 4–5 h [8]. This kind of release pattern is potentially useful for timed or site-specific drug delivery [9–11]. To avoid interference of other variables on mechanistic interpretation of the biphasic release, release tests have been performed restrictedly in distilled water at ignorable ionic strength. It is mandatory to investigate the effect of the influencing variables that might be possibly encountered both in vitro and in vivo on drug release characteristics.

The orally administered pectin-based matrix tablet will encounter a series of physiological pHs ranging from 1 to 7 or above when it is transported from the stomach down across the small intestine to the colon. Exposure to increasing pH will induce ionization and accelerated hydration of the pectin chains, which resultingly will affect the release behavior of pectin/calcium-based systems [12,13]. Furthermore, the gastrointestinal content contains a variety of ions at definite ionic strength among which cations may compete for the binding sites with calcium ions, thus potentially influencing the performance of pectin/calcium-modified matrix tablet [14,15].

Stability is another key point with the pectin/calcium matrix system. As the in situ crosslinking concept exploits the interaction of pectin and calcium upon contact with water, the dry matrix is latently reactive. It is rational to assume that the release profile as well as other properties of the matrix tablet undergoes a series of changes when it encounters hostile storage conditions such as elevated temperature, humidity and illuminance.

In this report, the effect of pH and salt concentration on biphasic release of indomethacin from HPMC/pectin/calcium chloride matrix tablet was investigated. Stability study with an emphasis on release behavior was performed under stress testing, accelerated and long-term storage conditions. Preliminary pharmacokinetic study was also performed in Beagle dogs to evaluate the effect of biphasic release in vivo.

2. Materials and methods

2.1. Materials

Micronized indomethacin (<5 μm) was purchased from Sine Pharmaceuticals (Shanghai, China). Pectin HM 70 (high methoxylated) and PVP K30 were kindly gifted from Shanghai Representative Office, ISP (Hong Kong) Ltd. Calcium chloride ($CaCl_2$) was of analytical purity and purchased from Shanghai Chemical Regent Corp. (Shanghai, China). Hydroxypropyl methylcellulose (HPMC, Methocel K4M) was a Dow Chemicals product and kindly gifted by Shanghai Colorcon Corp. Ltd. The commercial pectinase named Pectinex Ultra SP-L with an activity of approximately 10,000 pg/ml was provided by Novo Nordisk Ferment (Switzerland). All other chemicals were of analytical grade.

2.2. Preparation of HPMC/pectin/calcium hybrid matrix tablet

The matrix tablets were prepared following the same wet granulation procedures as in the previous study [8]. Each HPMC/pectin/calcium chloride tablet was mainly composed of indomethacin 25 mg, HPMC 100 mg, pectin 100 mg and calcium chloride 100 mg. First, all components were mixed homogeneously in a laboratory shaker-mixer. 10% (w/v) PVP K30 ethanol solution was added gradually and milled continuously to make paste. The wet mass was forced through a 20-mesh sieve and dried at 50 °C for 3 h. The dried granules were lubricated with magnesium stearate in 1% (w/w) and compressed into flat 10 mm tablets using a ZDY-8 model single punch compressor (Yuandong Pharmaceutical Machinery Co., Shanghai, China). The HPMC/pectin matrix tablet of the same composition but without calcium chloride was also prepared similarly as a reference for the pharmacokinetic study. The tablets were sealed in glass bottles before tests.

2.3. Release study

Release tests were performed following similar procedures as in previous study in 900 ml of release media thermostatically maintained at 37 ± 0.5 °C with a basket rotation speed of 100 rpm [8]. Tween 80 (0.2%, w/v) was added to the release medium to keep sink conditions. Citric buffer at similar ionic strength was used to evaluate the effect of pHs, and sodium chloride was used to increase the ionic strength in the release medium. To explore the potential of colonic enzyme on drug release, pectinase was also spiked to evaluate enzyme-triggered release. Exactly 5 ml of the sample was withdrawn at time intervals, filtered through 0.4 μm membrane (Millipore) and assayed by an Agilent 1100 series HPLC system [8]. An equal volume of the same release medium was supplemented to keep constant volume.

The power law Eq. (1) [16,17] was employed to describe the release kinetics, and the time of 10% ($T_{0.1}$), 50% ($T_{0.5}$) and 80% ($T_{0.8}$) drug release was also calculated from the correlation equations.

$$\frac{M_t}{M_\infty} = Kt^n \tag{1}$$

where M_t is the amount released at time t, M_∞ is the total amount released, K is a constant incorporating the properties of the macromolecular polymeric system and the drug, and n is the diffusion exponent that depends on the transport mechanism and the shape of the matrix tested.

2.4. Stability study

The stability of indomethacin HPMC/pectin/calcium chloride matrix tablets was evaluated under stress, acceler-

ated and long-term testing conditions according to the appendix method in Chinese Pharmacopoeia (2005 Ed.). Special attention was paid to changes in drug release characteristics.

2.4.1. Stress testing

The tablets were exposed to hostile temperature, humidity and light illumination at the following storage conditions: (1) 60 °C in a closed heater (Shanghai Jinghong Lab Equipment Co., China) without control of relative humidity (*RH*). (2) 92.5% *RH* at 25 °C in a closed heater. Saturated KNO_3 solution in hermetically sealed desiccators was employed to attain 92.5% *RH*. (3) 4500 ± 500 Lx illuminance at 25 °C attained by a Percival stability tester (Percival Scientific Inc., U.S.). The samples were collected and evaluated at 5 and 10 d.

2.4.2. Accelerated testing

The tablets were sealed in double aluminum foil and stored in a Percival stability tester maintained at 40 °C and 75% *RH*. At 1, 2, 3 and 6 month, the samples were collected and evaluated.

2.4.3. Long-term testing

The tablets in the same package were stored in a Percival stability tester maintained at 25 °C and 60% relative humidity. At 3, 6, 9, and 12 m, the samples were withdrawn and evaluated. Now, the samples are still in storage for further evaluation at 18, 24 and 36 m.

2.5. Determination of indomethacin in dog plasma

Indomethacin in dog plasma was determined by an HPLC method [18]. The Agilent 1100 series HPLC system was composed of a quaternary pump, a degasser, an autosampler, a column heater, and a tunable wavelength UV detector. The analysis was performed at 40 °C on a C18 column (Venusil XBP^M, 5 μm, 4.6 mm × 150 mm, Agela, China) guarded with a refillable precolumn (C18, 1.0 mm × 20 mm, Alltech, USA) using a mobile phase of acetonitrile and 0.1 mol/L sodium acetate (pH adjusted to 5.0 with acetic acid) in the ratio of 40/60 (v/v) pumped at a flow rate of 1.0 ml/min. The detection wavelength was 320 nm.

Plasma samples were prepared by liquid–liquid extraction. To 0.5 ml of plasma in a test tube, 0.1 ml of internal standard (an aqueous solution of naproxen in 10 μg/ml), 0.5 ml of 2% citric acid and 5 ml of diethyl ether were added. The mixture was vortex mixed for 5 min and centrifuged at 1600*g* for 15 min. The upper organic layer was transferred to a clean test tube and then evaporated under a stream of nitrogen at 40 °C. The dried residue was reconstituted in 0.1 ml of mobile phase and transferred to an autosampler vial for HPLC analysis.

2.6. Pharmacokinetic study

The pharmacokinetics of the HPMC/pectin/calcium chloride matrix tablet was compared with that of indomethacin powder and the HPMC/pectin matrix tablet in Beagle dogs in a randomized three-period crossover study after an oral dose of 25 mg equivalent of indomethacin. The washout period between administrations was 1 week. Six male Beagle dogs weighing 8–10 kg were kept in an environmentally controlled breeding room for 1 week before the start of the experiments. The dogs were fed standard laboratory chow with water and fasted overnight before the experiments. Guidelines on experiments involving use of animals issued by the Ethical Committee of Fudan University were strictly followed.

Indomethacin powder (25 mg) was diluted with lactose monohydrate (100 mg) and filled into a #3 hard gelatin capsule before administration. All of the three formulations were administered orally with 20 ml of water. At time intervals, 2 ml of blood samples was withdrawn into heparinized test tubes and centrifuged at 3000*g* for 10 min and stored at −20 °C until assay.

Pharmacokinetic analysis was performed by means of a model-independent method using 3p97 computer program (issued by the State Food and Drug Administration of China for pharmacokinetic study). C_{max} and T_{max} were observed as raw data. Lag time (T_{lag}) was calculated using method of residuals. Area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule.

3. Results and discussion

3.1. Release in phosphate buffer

Release of indomethacin from HPMC/pectin/calcium chloride matrix tablet was firstly evaluated in phosphate buffer because it is commonly used as release medium. Results indicated that release in pH 6.8 and 7.4 phosphate buffer was extremely limited with a total release percentage below 5% after 26 h. A layer of tight coating formed around the tablet and protected the matrix from erosion. The tablet can be observed in its original shape until the end of the release test. As calcium in the matrix was reactive to phosphate ions in the media to form precipitate, the coating might be a hybrid of the matrix material (pectin and HPMC) and calcium phosphate. As phosphate ions besides several other ions that are able to react with calcium to form insoluble salts also exist in the physiological media of the gastrointestinal tract, attention should be paid to their effect on in vitro and in vivo behavior of this matrix tablet.

Due to the above-mentioned reason, phosphate buffer is not suitable to be used as media to evaluate the release behavior of matrix that contains calcium. So, citrate buffer was used instead in this study.

3.2. Effect of pH of the release media

Release profiles of indomethacin from the matrix tablet at different pHs in HCl solution and citrate buffer are

shown in Fig. 1. It is evident that the pH of the release media has a significant influence on indomethacin release characteristics, especially the overall release rate, the biphasic release pattern (n and K value) and the initial release delay ($T_{0.1}$). An increasing tendency of the overall release rate was observed from pH 1.2 to 7.4. At pH 1.2 in 0.1 M HCl solution, the release profiles within the time range of 0.5–8 h kept biphasic characteristics, while the release rate was maintained at much lower level for a prolonged time of over tens of hours thereafter. At pH 3.0 and 5.5, release of indomethacin did not differ significantly before 10 h, and the release at pH 5.5 was just slightly faster than that at pH 3.0. After 10 h, release at pH 5.5 was much faster than that at pH 3.0. Overall release of indomethacin was much faster at pH 6.8 and 7.4 than at any other pHs both at initial and later stages.

When the release profiles were characterized by the power law, n values in the range of 1.1–1.4 with correlation coefficients of over 0.99 were observed, which is the most important character to define a matrix-based biphasic release profile. The correlation K, n and r values and the times for 10%, 50% and 80% release are shown in Table 1. There is an increasing tendency of n values from pH 1.2 to 7.4, which may allow for more desirable biphasic release as discussed previously [8]. Reflected in the release profiles, the pH increase resulted in only slight increase in the initial release rate and much faster release rate at later stage. Therefore, the initial release lag ($T_{0.1}$) did not vary significantly from pH 1.2 to 7.4 (2.9–3.8 h). At lower pH 1.2, 3.0 and 5.5, the $T_{0.1}$ was 3.2, 3.7 and 3.8 h, respectively. Favorable release lag may be envisaged when the matrix tablet is transported from the stomach down to the small intestine trespassing a pH gradient from 1 to 7.

The influence of pH on indomethacin release from the matrix lies in two aspects, i.e. the influence on the drug itself and the influence on the matrix material, especially pectin. Indomethacin, a methylated indole derivative and a member of the arylalkanoic acid class of NSAIDs, was practically insoluble at pH 1.2. Although Tween 80 has been added in the release medium as a solubilizer, the slow dissolution rate of indomethacin may contribute to the final release rate. Furthermore, stability study of indomethacin in 0.1 M HCl solution showed 30% percent degradation in 10 h at an original level of 2 μg/ml (data not shown). These factors contributed to the drop in release rate at later stages in 0.1 M HCl solution. However, it is strange that the initial release at pH 1.2 was faster than that at pH 3.0 and 5.5 with increased solubility and reliable stability of indomethacin. The variation in the physicochemical properties of the HPMC/pectin/calcium gel may provide an understanding. Lootens et al. [19] have studied the influence of pH on pectin/calcium strength and found that for non-amidated pectin lowering the pH below 3 leads to weakening of the gel formed by Ca^{2+}, which could possibly originate from a reduced affinity for Ca^{2+} at low pH. Similarly, the association of Ca^{2+} with the pectin HM 70 chain may also be weakened at extremely low pH of 1.2. Therefore, the in situ crosslinking stress on the hybrid hydrogel as a result of pectin/calcium interaction would not be as much as that at higher pHs and resulted in higher release rate at initial stages. At pH 3.0 and 5.5, adequate association of pectin and calcium assures tight controlling on indomethacin release, while at higher pHs of 6.8 and 7.4 the solubility of indomethacin increased greatly, diffusion may play a role in the release mechanisms in addition to erosion [8]. Moreover, pectin is an anionic biopolymer that readily undergoes ionization at elevated pHs and shows increased hydration and swelling as a result [1]. Therefore, the higher pHs were associated with faster erosion of the pectin matrix and faster indomethacin release that was solely controlled by erosion.

Table 1
Effect of release medium pH and salt concentration on the power law correlation parameters and time for 10% ($T_{0.1}$), 50% ($T_{0.5}$) and 80% ($T_{0.8}$) indomethacin release

	Power law correlation parameters				$T_{0.1}$ (h)	$T_{0.5}$ (h)	$T_{0.8}$ (h)
	Time span (h)	K	n	r			
Effect of pH							
1.2	0.5–8	0.02509	1.1996	0.9990	3.2	–	–
3	0.5–26	0.02054	1.2151	0.9995	3.7	13.8	20.4
5.5	0.5–20	0.01734	1.3239	0.9981	3.8	12.7	18.1
6.8	0.5–17	0.02096	1.3482	0.9990	3.2	10.5	14.9
7.4	0.5–14	0.02285	1.3818	0.9986	2.9	9.3	13.1
Effect of NaCl concentration (%)							
0	0.5–26	0.01573	1.2442	0.9973	4.4	16.1	>26
0.2	0.5–26	0.01646	1.2383	0.9975	4.3	15.8	>26
0.5	0.5–26	0.02071	1.1529	0.9955	3.9	15.8	>26
0.9	0.5–20	0.02333	1.2037	0.9977	3.4	12.8	18.9
2.0	0.5–17	0.03209	1.1786	0.9971	2.6	10.3	15.3
4.0	–	–	–	–	–	–	–

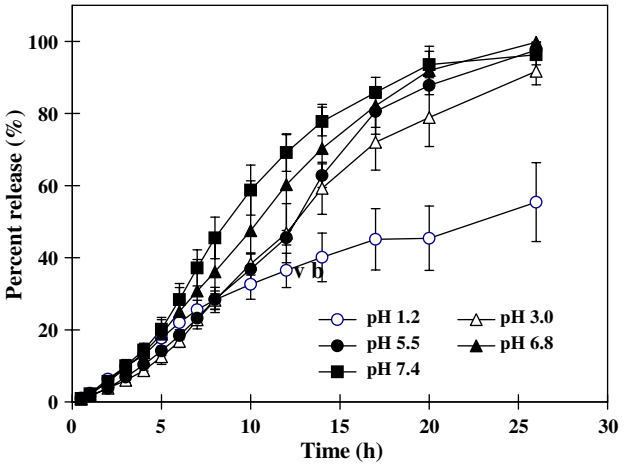

Fig. 1. Release profiles of indomethacin from the HPMC/pectin/calcium chloride matrix tablet in release media at different pHs (mean ± SD, $n = 3$).

3.3. Effect of salt concentration in the release media

The biphasic release characteristics are based on the interaction of anionic pectin chains and calcium ions. If the matrix tablet is administered orally, it certainly will encounter a series of physiological environments full of all kinds of positively and negatively charged ions. Since 0.9% NaCl solution was usually used to simulate physiological fluid, drug release in release media at a series of NaCl concentration around 0.9% was studied. Fig. 2 shows the release profiles of indomethacin in NaCl solutions and the power law equation fitting results are shown in Table 1.

Although model fitting showed increased K values and decreased n values and $T_{0.1}$ at higher NaCl concentration, the three release profiles at 0%, 0.2% and 0.5% NaCl levels did not differ significantly. When NaCl concentration increased to 0.9%, obvious increase in indomethacin release rate was observed. The initial stage release rate increased slightly with $T_{0.1}$ decreasing to 3.4 h, and the later stage release increased to much higher degree, which resulted in increased n value to over 1.20. At NaCl concentration of 2%, the overall release rate was significantly enhanced, but still kept a biphasic pattern. When extreme quantity of NaCl (4%) was used, release of indomethacin was enhanced significantly, which indicated possible disruption of the pectin/calcium associations.

Since indomethacin was practically insoluble in water, its release from matrices was usually controlled by erosion [7,8,20]. In the previous studies [7,8], release of indomethacin was found to correlate well with erosion of the matrices. Although the matrix erosion has not been studied here, the hybrid matrices were found to erode more quickly by visual observation when NaCl concentration was higher. Based on the previous studies on pectin matrix erosion [8], it is rational to propose an erosion-controlled release mechanism in the release media containing NaCl. The permeation of Na^+ into the HPMC/pectin/calcium matrix and concomitant interference with the pectin/calcium interaction resulted in decreased gel strength and thereby increased erosion and indomethacin release. Salts in pectin gels were found to change the pectin/Ca^{2+} gel texture and their viscoelastic properties [21], which may be ascribed to the decrease in cross-linking junctions between the pectin chains. The decrease is most considerable with increasing salt concentration or ionic strength in the solution. Similar interaction may also exist in this hybrid matrix and be employed to explain the effect of NaCl concentration on indomethacin release. In this hybrid matrix, several dynamic processes may take place when Na^+ competes with Ca^{2+} to bind to pectin chains resulting in certain release profiles. At lower level of Na^+, its effect was concealed because calcium in the matrix was in large amount, which was the reason that at NaCl concentration below 0.5% the release profiles did not differ significantly. When the ionic strength was big enough, the effect of Na^+ cannot be ignored. At NaCl level of 4%, the effect of Na^+ overwhelms that of Ca^{2+} after a small lag time. What is more interesting is the case with medium NaCl concentration, 0.9% and 2%. At initial stages, Ca^{2+} was greatly excessive which guaranteed sufficient association of calcium with pectin chains and the effect of Na^+ was not dominating. However, Ca^{2+} level in the matrix underwent quick decrease to a much lower level at later stages [8], and Na^+ overwhelmed Ca^{2+} to result in increased indomethacin release.

Then, it is concluded that the release behavior of indomethacin from the HPMC/pectin/calcium chloride matrix will not be influenced in environment of relatively lower ionic strength.

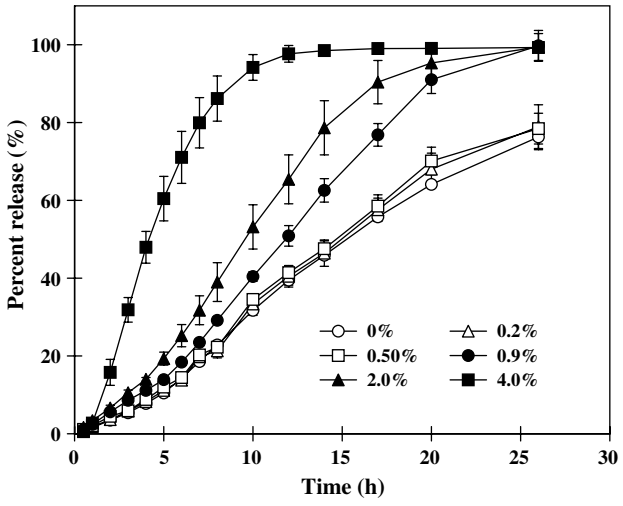

Fig. 2. Release profiles of indomethacin from the HPMC/pectin/calcium chloride matrix tablet in release medium at different sodium chloride concentrations (w/w) (mean ± SD, $n = 3$).

3.4. Release in gradient pH media and the effect of pectinase

The orally administered dosage form will transit across a pH gradient from the stomach to the colon, i.e. 0.5–2 h in stomach (pH 1–2) and 3–5 h in small intestine (pH 6–7) [22]. Study on the release in gradient pH media simulates the physiological pH of the gastrointestinal tract and provides useful information on the performance of the HPMC/pectin/calcium chloride matrix tablet under complicated release environment. Since the matrix tablet will surely arrive at proximal colon after a few hours, where it is to be degraded by local microflora, the effect of Pectinex Ultra SP-L on release characteristics was also studied. Fig. 3 shows the release profiles of HPMC/pectin/calcium chloride matrix tablet in gradient pH media (pH 1.2 HCl solution for 2 h, pH 7.4 citrate buffer for 3 h and pH 6.8 citrate buffer for 21 h). Although the matrix tablet will previously encounter pH 1.2 HCl solution and pH 7.4 citrate buffer in the first 5 h, the overall release profiles do not differ significantly from that in pH 6.8 citrate buffer.

When pectinase was added in the release media, obvious enzyme-triggered release was observed. After addition of pectinase at 5 h, pectinase in a concentration of

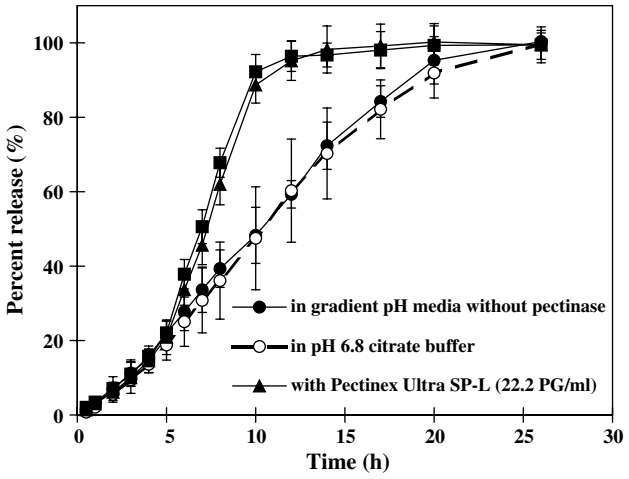

Fig. 3. Release profiles of indomethacin from the HPMC/pectin/calcium chloride matrix tablet in gradient pH release medium (0–2 h, pH 1.2 HCl solution; 2–5 h, pH 7.4 citrate buffer; >5 h, pH 6.8 citrate buffer) and with pectinase (mean ± SD, $n = 3$).

22.2 pg/ml induced dramatic increase in indomethacin release. The total release was approximately 90% at 10 h contrasting with about 45% release without pectinase. It is known that the pectin/HPMC hybrid coating is readily degraded by pectinase [23]. Here, the abrupt increase in indomethacin release may be also ascribed to the effect of pectinase-induced degradation of the hybrid matrix in this study. Therefore, the HPMC/pectin/calcium chloride is supposed to be sensitive to proximal colon microflora in vivo, thus be potentially colon-specific. Since the pectinase concentration was much smaller, we tested the enzyme-triggered release at double pectinase concentration of 44.4 pg/ml and found that there is only slight increase in the overall indomethacin release rate. It is understood that higher levels of enzymes may lead to faster degradation of the substrate matrix and thereby faster drug release rate [18]. However, for HPMC/pectin hybrid matrix its degradation may also depend on the erosion of the HPMC fraction that does not respond to pectinase, and when pectinase level was higher it may work as a rate-limiting factor, which served as the reason that there is no significant difference in the overall release rate at two different levels of pectinase concentration.

3.5. Stability

Under stress, accelerated and long-term testing conditions, the content of indomethacin did not change much (within 98.00–105.0% labeled content) indicating chemical stability of indomethacin. The appearance of the buffy round tablets also did not change much under all storage conditions except under high humidity conditions when the matrix took in water, swelled and became yellow. The overall weight gain was 46.5% and 77.9% at 5 and 10 d under 92.5% *RH* storage, respectively. This result indicated that the HPMC/pectin/calcium chloride matrix tablet takes in water easily due to the hygroscopic nature of pectin and calcium chloride suggesting possible influence on the pectin/calcium interaction and the release performance of the matrix tablet consequently. The stability study results are summarized in Table 2.

Release profiles and the power law correlation results after stress testing storage are shown in Fig. 4 and Table 2. There is no significant difference between the release profiles before storage and those after storage at 60 °C and 4500 ± 500 Lx illuminance. Similarity factors (f_2) of over 60% indicated similar release profiles [24,25] together with similar K and n values. However, increased release rate was observed after exposure to 92.5% *RH*, and the release rate at 10 d was even faster than that at 5 d. The f_2 values

Table 2
Stability study of HPMC/pectin/calcium chloride matrix tablets under stress, accelerate and long-term testing conditions

Stability testing conditions	Appearance	Weight (mg)	Content (%)	Power law correlation parameters			
				Time span (h)	K	n	r
Before testing	buffy, smooth	329.6	98.40	0.5–20	0.02438	1.2644	0.9985
Stress testing							
60 °C 5 d	buffy, spotted	325.1	98.82	0.5–20	0.02918	1.2057	0.9948
10 d	buffy, spotted	321.1	100.2	0.5–20	0.02867	1.2339	0.9958
92.5% *RH* 5 d	yellow, swelled	482.8	104.3	0.5–20	0.0393	1.1188	0.9952
10 d	yellow, swelled	586.5	101.4	0.5–20	0.04126	1.1232	0.9968
4500 ± 500 Lx illuminance 5 d	buffy, smooth	330.2	98.31	0.5–20	0.02352	1.2917	0.9983
10 d	buffy, smooth	326.5	98.65	0.5–20	0.02798	1.2144	0.9956
Accelerated testing							
1 m	buffy, smooth	325.1	98.82	0.5–20	0.02303	1.2974	0.9983
2 m	buffy, smooth	321.1	99.20	0.5–20	0.2518	1.2485	0.996
3 m	buffy, smooth	332.3	98.92	0.5–20	0.02311	1.2879	0.9978
6 m	buffy, smooth	321.1	98.33	0.5–20	0.02051	1.3388	0.9973
Long-term testing							
3 m	buffy, smooth	332.3	98.83	0.5–20	0.02277	1.2695	0.9962
6 m	buffy, smooth	325.1	99.27	0.5–20	0.02674	1.2397	0.9985
9 m	buffy, smooth	328.6	98.68	0.5–20	0.02643	1.2385	0.9965
12 m	buffy, smooth	330.5	99.54	0.5–20	0.02254	1.3058	0.9985

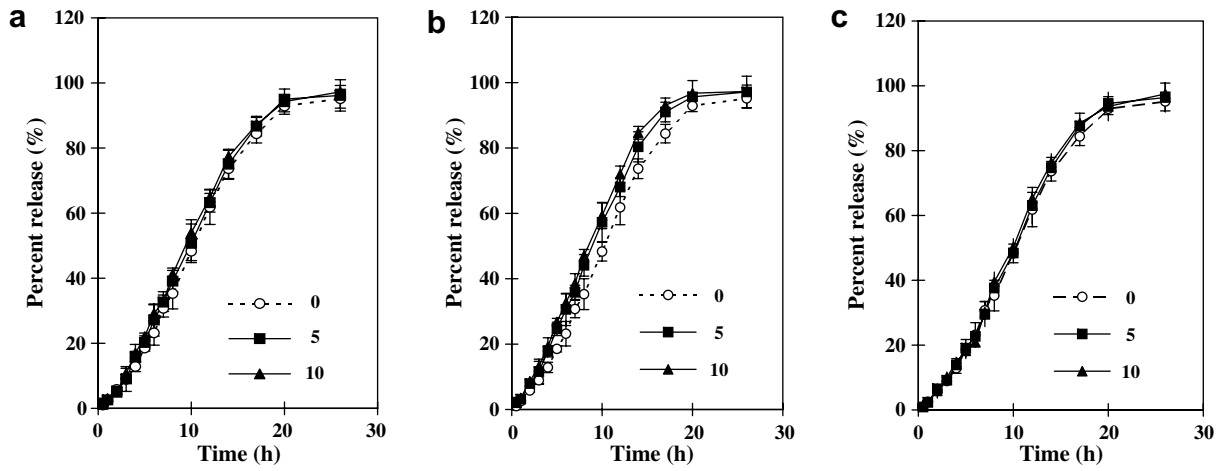

Fig. 4. Release profiles of indomethacin from the HPMC/pectin/calcium chloride matrix tablet in pH 6.8 citrate buffer after stress testing (a: 60 °C; b: 25 °C, 92.5 *RH*; c: 4500 ± 500 Lx illuminance) for 5 and 10 d (mean ± SD, $n = 3$).

between each two profiles of those before storage, after 5 and 10 d of storage were all below 50 indicating lack of similarity. Decrease in n values was observed due to enhanced release at initial stages which makes the release profiles approach zero-order kinetics, while increased K values suggested enhanced overall release rate.

Under accelerated testing and long-term testing conditions, the release profiles (not shown) did not change much. The f_2 values of over 60 confirmed similarity of the release profiles after storage under 40 °C/75% *RH* for 1, 2, 3 and 6 m and 25 °C/60% *RH* for 3, 6, 9 and 12 m.

It indicates that the stability of the HPMC/pectin/calcium chloride matrix is acceptable although pectin is potentially reactive to calcium. However, the release profiles were affected under extreme relative humidity of 92.5%, which suggested that hermetical package was desirable to protect the matrix tablet from taking in water.

3.6. Pharmacokinetics in Beagle dogs

The pharmacokinetics of the HPMC/pectin/calcium chloride matrix tablet was evaluated in Beagle dogs and compared with indomethacin powder and HPMC/pectin matrix tablet. Mean plasma indomethacin concentration versus time profiles after a single oral dose of the three formulations are shown in Fig. 5. Mean values of pharmacokinetic parameters such as T_{max}, C_{max}, AUC, T_{lag} and MRT are summarized in Table 3.

Being highly hydrophobic, indomethacin is readily absorbed along the whole alimentary tract. Indomethacin capsule showed the fastest absorption with the smallest T_{max} of 1.0 h and the highest C_{max} of 4192 ng/ml with a plasma indomethacin profile similar to a system that reported for improved dissolution [26] or fast-dissolving pellets [18].

Both the HPMC/pectin/calcium chloride and the HPMC/pectin tablets showed sustained release/absorption characteristics. Due to the difference in release characteristics [8], these two formulations showed difference in pharmacokinetic parameters. As learned from in vitro study, release of indomethacin from the HPMC/pectin/calcium

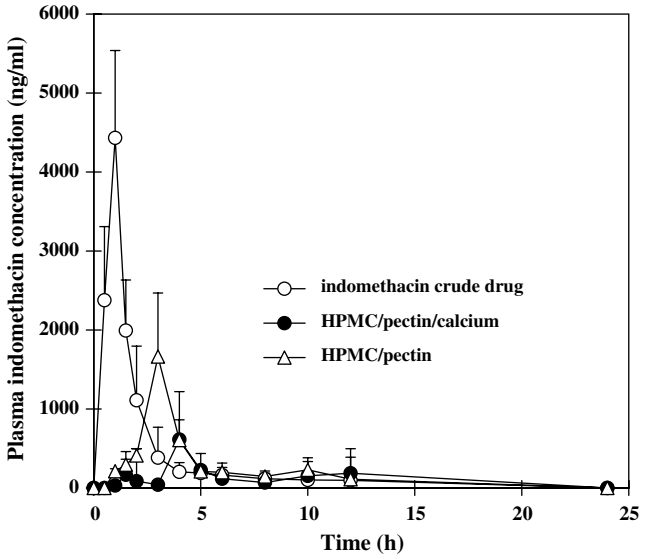

Fig. 5. Plasma indomethacin concentration versus time plot after a single oral dose of indomethacin crude drug, HPMC/pectin matrix tablet and HPMC/pectin/calcium chloride matrix tablet (mean ± SD, $n = 6$).

Table 3
Pharmacokinetic parameters of indomethacin following a single oral dose of three formulations in Beagle dogs (mean ± SD, $n = 6$)

	HPMC/pectin/calcium chloride matrix tablet	Indomethacin crude drug	HPMC/pectin matrix tablet
T_{lag} (h)	0.80 ± 0.24	0	0.48 ± 0.09
$T_{1/2}$ (h)	7.91 ± 3.31	3.37 ± 0.18	3.45 ± 1.02
MRT (h)	7.13 ± 0.42	3.97 ± 2.20	5.61 ± 3.00
T_{max} (h)	4.00 ± 0.00	1.00 ± 0.00	3.00 ± 0.00
C_{max} (ng/ml)	604 ± 206	4192 ± 1103	1662 ± 438
AUC_{0-t}(ng/h/ml)	2167 ± 709	7574 ± 1910	4319 ± 1035

chloride tablet followed biphasic pattern, typically slow at initial stages, which contributes to the low level of indomethacin in plasma within a few hours after drug administration. As observed, the plasma indomethacin level was maintained at a much lower level until 3 h. The absorption lag time for HPMC/pectin/calcium matrix tablet was about 0.8 h, which is a little longer than 0.4 h for HPMC/pectin matrix tablet. However, the absorption lag time was shorter than what we have observed for guar gum/Eudragit FS double coated indomethacin pellets [18]. As the matrix is an open system that is ready to release its load once it contacts with water, it is impossible to prevent drug release at its earlier stages. Although the pectin/calcium interaction exerts sufficient retardation on indomethacin release from the matrix, some of the drug may have released and been absorbed before arriving at colon. Consequently, there seems to be a sustained low level of indomethacin in the beginning 3 h for the in situ crosslinking system.

The T_{max} of HPMC/pectin/calcium and HPMC/pectin matrix tablet was 4 and 3 h, respectively, and the former showed much lower C_{max} of 604 ng/ml than 1662 ng/ml for the latter. This is in accordance with in vitro release behavior that HPMC/pectin release faster than the in situ crosslinking matrix tablet. Taking into consideration that release of indomethacin from HPMC/pectin/calcium matrix tablet was below 10% within 5 h, the T_{max} in this study seemed to be shorter than expected. As discussed in the previous study [18], pharmacokinetic data in Beagle dogs may differ from that commonly accepted for human beings because the canine species always showed faster transit time [27]. When the HPMC/pectin/calcium matrix tablet transits down to the colon at about 3 h, enzymes of the colon immediately trigger degradation of the matrix and resultant release and absorption of indomethacin.

The *MRT*s (mean resident time) for indomethacin crude drug, HPMC/pectin and HPMC/pectin/calcium matrix tablets were 3.97, 5.61 and 7.13 h, respectively, which in another aspect confirmed the delayed absorption of the in situ crosslinking matrix tablet as a result of retardation on initial drug release. The *AUC* of indomethacin crude drug was similar to previous report [18], while that of the HPMC/pectin/calcium or HPMC/pectin matrix tablets was only about 1/4 or 1/2, respectively. Since indomethacin release from the HPMC/pectin matrix in vitro was over 80% at 12 h [8], complete release in vivo can be expected too. There is sufficient time for indomethacin release from the HPMC/pectin matrix tablet as the resident time of a dosage form in colon may extend to tens of hours. Furthermore, enzymatic degradation may also add speed to in vivo release of the matrix tablet. Therefore, incomplete absorption should be regarded as the main reason for reduced *AUC*. For the HPMC/pectin/calcium matrix tablet, the *AUC* was only half that of the HPMC/pectin tablet. Besides incomplete absorption, incomplete and significantly delayed release of indomethacin as a result of in situ crosslinking may also contribute to this situation.

In general, the results of in vivo evaluation showed that the in situ crosslinking HPMC/pectin/calcium matrix tablet could provide sufficient time delay, which may be related with more effective delivery of drugs to the colon.

4. Conclusions

The pH of the release medium has a significant influence on release characteristics. From pH 1.2 to 7.4, an increasing tendency of the overall release rate and the power law correlation n values was observed. However, the 10% release time ($T_{0.1}$) did not vary much showing favorable release lag. Ionic strength also influences the biphasic release characteristics at sodium chloride levels in the media over 0.5%. Obvious increase in overall release rate was observed at sodium chloride level of over 0.9%. The hybrid matrix has sufficient sensitivity to pectinex Ultra SP-L indicating obvious triggering on drug release. The HPMC/pectin/calcium chloride matrix was stable under accelerated and long-term testing conditions, but stress testing indicated that the release characteristics was affected by high relative humidity. Pharmacokinetics in dogs indicated that the in situ crosslinking HPMC/pectin/calcium matrix tablet could provide sufficient time delay, which may be related with more effective delivery of drugs to the colon.

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