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Mechanistic Analysis of Drug Release from Theophylline Pellets Coated by Films Containing Pectin, Chitosan and Eudragit® RS

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The objective of this study was to obtain detailed information on the mechanism of drug release from mixed-film of pectin-chitosan/Eudragit® RS. Pellets (710–840 μm in diameter) containing 60% theophylline and 40% microcrystalline cellulose were prepared by extrusion-spheronization method. Eudragit® L100-55 enteric coating capsules included film-coated pellets of theophylline in theoretical coating weight gains of 10, 15, and 20%, with pectin-chitosan complex contents of 5, 10, 15, and 20% for each level of weight gain were prepared and subjected to in vitro drug release. Drug release from this system showed a bimodal release profile characteristic with the drug release enhancement, being triggered (burst release) in the colonic medium. The reason for burst drug release may be due to the enzymatic degradation of pectin via pectinolytic enzymes in the simulated colonic medium. The mechanism of drug release from each formulation was evaluated in the terms of zero-order, first-order, Higuchi and Korsmeyer-Peppas models. It was observed that none of the enteric coating capsules showed any drug release in the simulated gastric medium (phase I). The analysis of release profiles showed that zero-order kinetics was found as the better fitting model for all formulations in the simulated small intestine (phase II) and it could be due to the pectin-chitosan swelling and subsequent formation of aqueous channels. In the colonic medium (phase III), due to degradation of pectin and its leaching from the mixed-film, there was a modification in drug release kinetics from swelling-controlled at phase II to anomalous at phase III. It also was found that both zero-order and Higuchi models contributed in colonic drug release from most of the formulations.

Keywords bimodal drug delivery; burst drug release; chitosan; drug release kinetics; Eudragit® RS; mixed-film; pectin

INTRODUCTION

For decades, polymeric systems have been used for pharmaceutical applications, especially to provide controlled release of drugs. Bimodal or sigmoidal drug release profiles, where release is slow in the initial stages and increases to a faster release rate at some later stages, may be of significant therapeutic benefit (Maggi & Conte, 1997). In disease states such as nocturnal asthma, incremental release rate may be helpful to prevent exacerbation of nocturnal or early symptoms caused by circadian rhythms (Lemmer, 1991). Bimodal release profiles could be utilized so that drug release was slower in a region within the gastrointestinal tract (GIT) where absorption is good (i.e., small intestine) and increased lower down the GIT where

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drug absorption may be poor (i.e., colon). The overall effect would be to maintain therapeutic blood drug levels throughout (Macleod et al., 1999a). Colonic delivery of drugs through bimodal release mechanisms is aimed at treating local diseases of the colon or maintaining sustained blood drug levels (Macleod et al., 1999b). Several approaches developed for the purpose of achieving colonic/bimodal drug delivery include time-controlled (Hebden et al., 1999; Steed et al., 1997), pH-dependent (Cole et al., 2002; Markus et al., 2001), pressure-controlled (Muraoka et al., 1998; Shibata et al., 2001), and microflora-triggered delivery systems (Brondsted, Andersen, & Hovgaard, 1998; Katsuma et al., 2002; Yano et al., 2002). Bimodal release is usually observed in press-coated or film-coated systems with tablets or pellets as substrate. This kind of release pattern needs triggering mechanisms such as degradation of the polysaccharide coating by colonic microbial degradation (Krishnaiah et al., 2002) or organic acid-induced enhancement of drug release (Narisawa et al., 1994).

Carbohydrate polymers, such as pectin and chitosan, have been widely employed in the pharmaceutical formulations, especially colon-specific drug delivery (Ashford et al., 1994; El-Gibaly, 2002; Koizumi, Ritthidej, & Phaechamud, 2001; Macleod, Fell, & Collett, 1997; Macleod et al., 1999a, 1999b; Munjeri, Collett, & Fell, 1997; Murata et al., 2004; Ofori-Kwakye & Fell, 2001, 2003; Sriamornsak & Nunthanid, 1998; Sriamornsak et al., 1997). Recently, pectin polymer has been used as a pore former in polymeric membrane for colonic targeting of 5-fluorouracil (Wei et al., 2007). Moreover, a microbially triggered colon-targeted osmotic pump (MTCT-OP) has been designed based on chitosan degradation by microflora of the colon and thereby in situ formation of a microporous membrane (Liu et al., 2007).

Electrostatic interaction between pectin (polyanion) and chitosan (polycation), lead to the formation of polyelectrolyte complex (PEC) which can provide a greater barrier to drug release in the upper GIT than either polymer alone (Munjeri et al., 1997). Mixtures of pectin and chitosan have been used as compression coatings (Fernández-Hervás & Fell, 1998) and film coatings (Ofori-Kwakye & Fell, 2003). The major problem encountered with polysaccharides, such as pectin and chitosan, is their high water-solubility and swelling properties in aqueous media. Films consisting of pectin and chitosan are unable to prevent the fast drug release during the transit through the stomach and small intestine. However, the incorporation of these hydrophilic polysaccharides in water-insoluble film-forming polymers such as cellulosic or acrylic polymers could provide a promising alternative (Semdé, Amighi, Devleeschouwer, & Moës, 2000a, 2000b).

In our previous study, the potential of Eudragit® RS, containing various amounts of pectin-chitosan ionic complex (as coating material) in several coating weight gains, intended for bimodal drug release from multiple-units (pellets) of theophylline was evaluated. Theophylline was used as a model water-soluble drug. In that earlier work, the influences of two mentioned factors on drug release profiles have been demonstrated without any quantitative treatment on release data (Ghaffari et al., 2006).

Concerning the mathematical modelling of drug release from controlled release systems, one must identify the most important transport phenomenon for the investigated system and neglect the other processes; otherwise, the mathematical model becomes too complex for easy use. The drug release models proposed in the majority of literatures were classified into four distinct models: zero-order, first-order, Higuchi, and Korsmeyer-Peppas model. The mechanism of drug release associated with pectin-chitosan/Eudragit® RS mixed-film has not been described in the literatures yet. Thus, the purpose of this study was to elucidate the mechanism of drug release from the mentioned system. In the current study, drug release mechanisms are characterized using mathematical fitting of release data into the four aforementioned models.

MATERIALS AND METHODS

Materials

Pectin (from citrus fruit, methoxy content = 8.9%, galacturonic acid content = 88.2%) and chitosan (degree of deacetylation = 86.1%, viscosity of 1% w/v solution in acetic acid 1% v/v = 34 mPas) were employed as two components of mixed-film (Sigma-Aldrich Co., Dorset, UK). Eudragit® RS30D was kindly donated by Röhm Pharma (Darmstadt, Germany) and used as another ingredient for mixed-film fabrication. Eudragit® RS30D is a 30% w/w aqueous latex of poly (ethylacrylate methylmethacrylate trimethylammonioethyl methacrylate chloride). Theophylline anhydrous was supplied by BASF (Ludwigshafen, Germany) and used as a model drug. Eudragit® L100-55 also was donated by Röhm Pharma as an enteric-coating material. Microcrystalline cellulose (Avicel® PH 101) was supplied by FMC Corporation (Philadelphia, USA). Pectinex® Ultra SP-L (pectinolytic enzymes, extracted from *Aspergillus Niger*) was provided by Novo Nordisk Ferment (Dittingen, Switzerland). Other chemicals employed in this study included acetic acid, hydrochloric acid, sodium hydroxide, and triethyl citrate (Merck, Darmstadt, Germany). Distilled water was freshly prepared.

Preparation and Coating of Theophylline Pellets

The preparation and coating of theophylline pellets were discussed in detail in our previous work (Ghaffari et al., 2006). Briefly, theophylline pellets were prepared by the extrusion-spheronisation method. A 100-g batch of theophylline anhydrous and microcrystalline cellulose (Avicel® PH 101), in a weight ratio of 6:4 was mixed and kneaded in a planetary mixer (Kenwood Chef, UK) and then loaded into the extruder (type E-35, Gabler GmbH, Germany). Then, extrudates were immediately transferred to a spheroniser (type R-250, Gabler GmbH, Germany) equipped with a cross-hatch plate. Pellets were collected and dried in an oven at

50ºC for 12 hours after which sieve analysis was done and the fraction of 710–840 μm was separated for coating. In order to prevent the batch-to-batch variability of the pellets from affecting the different batches of coated pellets, the sieve-cuts of 710–840 μm from several batches of theophylline pellets were pooled together, blended, and then the pellets for coating were taken out from this bulk. For coating, theophylline pellets were coated with a combination of Eudragit® RS and pectin-chitosan PEC in a fluidized-bed coater (Uni-Glatt, Glatt GmbH, Germany) assisted by a Wurster column. After coating, the coated pellets were gently fluidized for about 5 min after which they were cured at 50ºC for 12 hours. The composition of each formulation is presented in Table 1. As can be seen in Table 1, different amounts of pectin-chitosan and Eudragit® RS were used as main components of the coating solution and mixed-film. Triethyl citrate was used as a plasticizer in all formulations (10% w/w related to the solid content of Eudragit® RS30D).

In our previous investigation (Ghaffari et al., 2006), it was observed that isolated (free) mixed-film of pectin-chitosan /Eudragit® RS dissolved rapidly in the acidic medium, while they swelled slowly and showed no dissolution in two phosphate buffer solutions. It can be assumed that pectin-chitosan complex in the mixed ternary blends dissolved and leached from the mixed-film coatings. Therefore, for bimodal drug delivery, an additional outer enteric-coating was necessary to prevent the drug release from coated pellets in the stomach. Accordingly, after capsules were filled with coated pellets, the capsules were enteric-coated by the alcoholic solution of Eudragit® L100-55 in a conventional coating pan (100 mg of Eudragit® L100-55 per capsule).

Determination of Drug Content (Assay) and Release

Drug contents of the uncoated and coated pellets were determined by HPLC analysis (US Pharmacopeia, 2005). Drug release studies were conducted using USP Apparatus I (basket) at 100 rpm and 900 mL of dissolution medium which was maintained at 37 ± 0.5ºC. Three capsules filled with each formulation (equivalent to 200 mg of theophylline) were tested individually in 0.1 mol/L HCl (pH 1.5) for first two hours (phase I), phosphate buffer solution pH 7.4 for the second three hours (phase II), and phosphate buffer solution pH 6.0 containing pectinolytic enzymes (4 mL/L) for the last five hours (phase III). At the end of each phase, the dissolution medium was replaced completely by another one. These media were chosen to mimic the conditions in the stomach, small intestine, and colon, respectively. At predetermined time intervals, 5 mL of samples were withdrawn and filtered (Millex® AP, Millipore, 0.4 μm). The filtrate was diluted and then analyzed spectrophotometrically at 271.8 nm. Meantime, an equal volume of the same medium was added to keep constant volume.

Analysis of Release Profile Data

Pectin-chitosan/Eudragit® RS coated pellets could be regarded as a biodegradable delivery system. Mathematical drug release modelling of this system is not as advanced as the modelling of diffusion or swelling-controlled devices, because it is generally more complex. Yet, no mathematical model has been described in the literature that accurately takes into account all the important processes involved to quantify drug release from biodegradable systems (Siepmann & Göpferich, 2001). Thus, in the current study, only models used for diffusion or swelling-controlled system were considered. In order to analyze the drug release mechanism, the following mathematical models or expressions were used as explained briefly below.

Zero-Order Kinetics

This model can be expressed by:

$$Q_t = Q_0 + K_0 t \tag{1}$$

where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$) and K_0 is the zero-order release constant. In this way, a graphic of the amount of dissolved drug versus time will be linear (Costa & Lobo, 2001).

First-Order Kinetics

The following equation can express this release kinetics:

$$\log Q_t = \log Q_0 + K_1 t/2.303 \tag{2}$$

where Q_t is the amount of drug released in time t, Q_0 is the initial amount of drug in the solution and K_1 is the first-order release constant. In this way, a graphical representation of the decimal logarithm of the released amount of drug versus time will be linear (Costa & Lobo, 2001).

Higuchi Model

Higuchi (1961, 1963) developed several theoretical models to study the release of water-soluble and low soluble drugs incorporated in semi-solid and/or solid matrices. In a general way, it is possible to resume the Higuchi model to the following expression (generally known as the simplified Higuchi model):

$$Q_t = K_H t^{1/2} \tag{3}$$

where Q_t is the amount of drug released in time t and K_H is the Higuchi release constant. Higuchi describes drug release as a diffusion process based on the Fick's law, square root time dependent.

TABLE 1
Composition of F_1–F_{12} Formulations

Formulation Code	Eudragit® RS (Solid Content, g)	Eudragit® RS30D (g)	Pectin – Chitosan Complex (g)	Triethyl Citrate (g)	Water ad (g)	Pectin – Chitosan Complex in Film (%, w/w)	Theoretical CWG[a] (%, w/w)	Actual CWG (%, w/w)[b]
F_1	9.50	31.67	0.5	0.95	285	5	10	9.8 ± 0.2
F_2	9.00	30.00	1.0	0.90	270	10	10	9.5 ± 0.1
F_3	8.50	28.33	1.5	0.85	255	15	10	8.7 ± 0.6
F_4	8.00	26.67	2.0	0.80	240	20	10	8.9 ± 0.4
F_5	14.25	47.50	0.75	1.42	427.5	5	15	14.1 ± 0.2
F_6	13.50	45.00	1.5	1.35	405	10	15	13.5 ± 0.8
F_7	12.75	42.50	2.25	1.27	382.5	15	15	13.8 ± 0.5
F_8	12.00	40.00	3.0	1.20	360	20	15	13.3 ± 0.2
F_9	19.00	63.33	1.0	1.90	560	5	20	18.6 ± 0.1
F_{10}	18.00	60.00	2.0	1.80	540	10	20	18.1 ± 0.9
F_{11}	17.00	56.67	3.0	1.70	510	15	20	19.0 ± 0.3
F_{12}	16.00	53.33	4.0	1.60	480	20	20	17.9 ± 1.2

[a]CWG: coating weight gain.
[b]Mean ± *SD*, $n = 3$.

Korsmeyer–Peppas Model

Korsmeyer et al. (1983) developed a simple, semiempirical model, relating exponentially the drug release to the elapsed time (t) (Costa & Lobo, 2001):

$$M_t/M_\infty = k_{KP}\, t^n \tag{4}$$

where k_{KP} is a constant, incorporating structural and geometrical characteristic of the drug dosage form, n is the release exponent, indicative of the drug release mechanism, and the function of t is M_t/M_∞ (fractional release of drug). The last equation is so-called power law.

Different values of n for cylindrical, spherical, and slab geometries are available in the literature. Ritger and Peppas (1987a, 1987b) used these values in order to characterise different release mechanisms. In the current study, the coated pellets were assumed as a sphere. For spheres, values of 0.43, $0.43 < n < 0.85$, and 0.85 are related to Fickian diffusion (case-I transport), anomalous, and "case-II transport," respectively (Table 2). When the exponent n takes a value of 0.85, the drug release rate is independent of time. This case corresponds to zero-order kinetics. The mechanism that creates the zero-order release is known as case-II transport. In case-II, the relaxation process of the macromolecular chains occurring upon water imbibition into the system is the rate-controlling step.

Korsmeyer-Peppas model is generally used to analyze the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than one type of release phenomena could be involved (Costa & Lobo, 2001).

Different mathematical models may be applied for describing the kinetics of the drug release process; the more suited model being the one that better fits the experimental results. The kinetics of theophylline release from different formulations was determined by finding the better fitting of the dissolution data into different models: 1–4, for zero-order, first-order, Higuchi, and Korsmeyer-Peppas models, respectively.

Direct fitting of the drug release data to the non-linear Korsmeyer-Peppas equation described above is usually avoided by performing a linear transformation of the data, followed by regression analysis. Nevertheless, this method may not be mathematically accurate, as it uses transformed values (logarithms) instead of the original data (Lu, Abu-Izza, & Chen, 1996). Therefore, a direct non-linear fitting of the experimental results was carried out in the present work.

TABLE 2
Exponent n of the Power Law and Drug Release Mechanism from Polymeric Controlled Delivery Systems of Sphere (Ritger & Peppas, 1987a, 1987b)

Release Exponent (n)	Drug Transport Mechanism
0.43	Fickian diffusion
$0.43 < n < 0.85$	Anomalous transport
0.85	Case-II transport
> 0.85	Super case-II transport

RESULTS AND DISCUSSION

Influence of the Coating Weight Gain and Pectin-Chitosan Content on Drug Release from Coated Pellets

The effects of two above variables on the drug release profiles were investigated in the F_1–F_{12} formulations (i.e., theoretical coating weight gains of 10, 15, and 20%, with pectin-chitosan complex contents of 5, 10, 15, and 20% for each level of weight gain). The drug release profiles obtained from enteric-coated capsules of F_1–F_4 formulations with 10% w/w of theoretical coating weight gain and different amounts of pectin-chitosan (5, 10, 15, and 20% w/w, related to the total amount of mixed-film for F_1–F_4, respectively) are presented in Figure 1. No drug release was observed over 120 minutes in pH 1.5 due to the protective enteric coating with Eudragit® L100-55. After the media change to pH 7.4, drug release started to occur. At phase III, phosphate buffer solution pH 7.4 was replaced completely by phosphate buffer solution pH 6.0, which is included 4 mL/L of pectinolytic enzymes. Pectinex® Ultra SP-L is a commercially available mixture of specific enzymes and its pectinolytic activity has shown to be closely correlated with that of the *Bacteroides Ovatus*, the main colon producer of pectinolytic enzymes (Wakerly, Fell, Attwood, & Parkins, 1996). For the first 10 min of the drug release study at third medium, release rates from all of the formulations were remarkably increased and it might be influenced by degradation of pectin by pectinolytic enzymes. Pectin undergoes a fast degradation process, which is in agreement with the results of release studies causing a more marked increase in the release rate. In brief this drug delivery system delivers no drug under

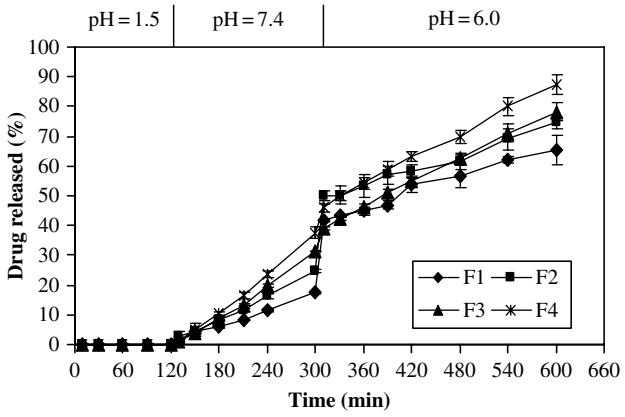

FIGURE 1. Cumulative percent of drug release versus time profiles for enteric-coated F_1–F_4 formulations. The vertical lines on the upper x-axis denote the time points for the changes in dissolution media (Mean ± *SD*, $n = 3$).

in vitro conditions that simulate the stomach (pH 1.5), commences the release of drug at moderate rates in conditions simulating the small intestine (pH 7.4), and accelerates the release rate in the presence of pectinolytic enzymes (pH 6.0). Such a system would be proper in a situation where a bimodal drug release profile was sought.

The pectin-chitosan complex in mixed-film is swellable and degradable. For F_1–F_4 formulations, the drug release rates (slopes) were different, depending on the pectin-chitosan fraction incorporated in the formulation. Drug release rates from these formulations were in the order of $F_4 > F_3 > F_2 > F_1$ (Figure 1). It was found that the higher amounts of pectin-chitosan, the more drugs released. In Table 3, the fitting results of the experimental drug release data into different kinetics equations for F_1–F_4 formulations are summarised. As can been seen, fitting analysis was performed for each point under release studies, with the exception of 300–310 min. At this time span, there was a burst drug release, due to changing in the diffusional conditions of drug release; above-mentioned kinetics models should not be used (Huang & Brazel, 2001; Ritger & Peppas, 1987b). Therefore, in the current study, segmental fitting analysis (e.g., 150–300 min and 310–600 min) was performed.

For F_1–F_4 formulations, at phase II of drug release, n parameter resulted from the Korsmeyer-Peppas equation was determined to be equal to 0.8046, 0.9683, 1.2079, and 1.1171, respectively (shown in Table 3). These values point to an anomalous (non-Fickian) diffusion mechanism for F_1 and super case-II transport for the others. For all of these formulations, zero-order kinetics yielded better quality adjustment.

At phase III of drug release, n values obtained from F_1–F_4 formulations were equal to 0.5111, 0.4337, 0.7488, and 0.6818, respectively. Values of n for F_1 and F_2 formulations indicated anomalous and purely Fickian diffusion, respectively; Higuchi model is more correlated for both of them (especially for F_2). In F_3 and F_4 formulations, n values pointing to an anomalous mechanism and both zero-order and Higuchi model characterized good quality adjustments. It was verified that Equation 4 has two distinct physical realistic meanings in the two special cases of n = 0.43 (indicating diffusion-controlled drug release) and n = 0.85 (indicating swelling-controlled release). Values of n between 0.43 and 0.85 can be regarded as an indicator for the superposition of both phenomena (anomalous transport). Values for the exponent n greater than 0.85 means that the drug release kinetics are predominantly swelling-controlled release (super case-II). According to Table 3, the increasing of pectin-chitosan fraction from F_1 to F_4 tended to change the drug release mechanism from anomalous to super case-II transport during phase II. In other words, hydration-swelling characteristic of pectin-chitosan may cause drug release kinetics to change. In the F_2, F_3, and F_4 formulations, swelling-controlled was predominant but in the first one there was a superposition of both swelling and

TABLE 3
Model Fitting of the Theophylline Release from F_1–F_4 Formulations

		F_1	F_2	F_3	F_4
Zero-order (phase II)	K_0 (mg/min)	0.1801	0.2745	0.3728	0.4359
	R^2	**0.9999**	**0.9971**	**0.9976**	**0.9974**
Zero-order (phase III)	K_0 (mg/min)	0.1721	0.1701	0.2702	0.2824
	R^2	0.968	0.9871	**0.9991**	**0.9979**
First-order (phase II)	K_1 (1/min)	0.0097	0.0113	0.0138	0.0129
	R^2	0.9848	0.9508	0.9163	0.9392
First-order (phase III)	K_1 (1/min)	0.0016	0.00014	0.0023	0.0021
	R^2	0.9683	0.9895	0.9853	0.9863
Higuchi (phase II)	K_H (mg/min$^{1/2}$)	3.3563	5.1528	7.002	8.1751
	R^2	0.9466	0.9675	0.9691	0.9662
Higuchi (phase III)	K_H (mg/min$^{1/2}$)	6.145	6.2289	9.6384	10.067
	R^2	**0.9794**	**0.9908**	**0.998**	**0.9959**
Korsmeyer-Peppas (phase II)	K_{KP} (1/min^n)	0.024	0.016	0.06	0.011
	n	0.8046	0.9683	1.2079	1.1171
	R^2	0.9723	0.9959	0.9988	0.9987
Korsmeyer-Peppas (phase III)	K_{KP} (1/min^n)	0.028	0.0498	0.077	0.0129
	n	0.5111	0.4337	0.7488	0.6818
	R^2	0.9756	0.975	0.9996	0.9979

Amount of drug release and time expressed in mg and min, respectively; R^2 is the coefficient of determination.

Better results of R^2 are in bold.

K_0, K_1, K_H and K_{KP} are the release rates calculated from zero-order, first-order, and Higuchi and Korsmeyer-Peppas models, respectively.

diffusion-controlled mechanisms. Similar *n* values of larger than 1.0 have also been reported by other authors (Alur et al., 1999; Ferrero, Muñoz-Ruiz, & Jiménez-Castellanos, 2000; Munday & Cox, 2000; Ranga Rao, Padmalatha Devi, & Buri, 1988). In these reports, the *n* values higher than 1.0 was ascribed to a super case-II transport, in which drug release would seem to be controlled by polymer relaxation/swelling (Korsmeyer et al., 1983). In the current study, Korsmeyer-Peppas model was employed just as a decision making tool, for example, between Higuchi and zero-order model and its parameter (*n*) could be invoked to further support the results obtained from the other models.

In general, due to degradation of pectin and leaching from the mixed-film during phase III, there was a modification in drug release kinetics from swelling-controlled at phase II to anomalous or pure Fickian diffusion (F_2) at phase III. All four formulations showed lower *n* values at the third phase as compared with the second phase and this phenomenon might be related to a considerable burst drug release at the beginning of phase III.

Figure 2 shows the curves of drug release from enteric-coated F_5–F_8 formulations. These formulations contain 15% w/w of theoretical coating weight gain and different amounts of pectin-chitosan (5, 10, 15, and 20% w/w, related to the total amount of mixed-film for F_5–F_8, respectively). Over again, the Eudragit® L100-55 enteric coating capsules did not dissolve in phase I. As can be seen in Figure 2, the trend of drug release is the same as in Figure 1, with the exception that F_5 and F_6 formulations do not show a burst release in phosphate buffer pH 6.0. The lower amounts of pectin-chitosan incorporated in F_5 and F_6 formulations as compared with F_7 and F_8 might be responsible for these differences in the release profiles. The amounts of pectin-chitosan available in F_5 and F_6 formulations were insufficient to make burst release in the colonic environment. Therefore, drug release from F_5 and F_6 was not bimodal and a slightly bimodal release characteristic could be observed for F_7 and F_8 formulations.

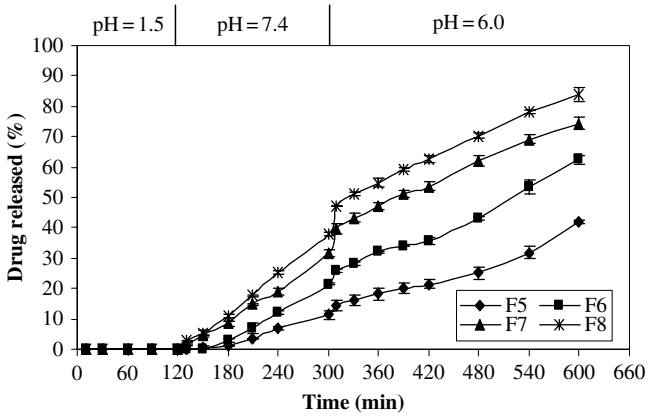

FIGURE 2. Cumulative percent of drug release versus time profiles for enteric-coated F_5–F_8 formulations. The vertical lines on the upper x-axis denote the time points for the changes in dissolution media (Mean ± *SD*, *n* = 3).

The fitting results of the experimental release data to different kinetics for F_5–F_8 formulations are presented in Table 4. During phase II, *n* values were determined to be equal to 1.8632, 2.1407, 1.1075, and 1.1088, respectively (Table 4). Unexpectedly, these values point to super case-II transport for these formulations. In addition, zero-order kinetics yielded better quality adjustment. Between 310 and 600 min (phase III), *n* values resulted from F_5–F_8 formulations were equal to 1.0644, 0.9235, 0.6741, and 0.6195, respectively. Values of *n* for F_5 and F_6 formulations indicated super case-II; first-order kinetics was more correlated for both of them. In F_7 and F_8 formulations, *n* values point to an anomalous mechanism and both zero-order and Higuchi model characterized good quality adjustments.

If we compare *n* values obtained from F_3–F_4 with F_7–F_8, it could be seen that there were no difference between the drug release kinetics during the release studies. While, the drug release mechanisms related to F_1 and F_2 were quite differed from F_5 and F_6. It might be pointed that due to no burst release in F_5 and F_6 formulations, release mechanism in the colonic medium was controlled by a first-order model, suggesting that the release was dependent on the amount of drug remaining in the pellet. It would seem that a balance between coating weight gain and pectin-chitosan amount might be included for the burst phenomenon in F_1 and F_2. Substantial amount of pectin-chitosan could induce the hydration and appearance of distensions in the mixed-film. Due to higher region of aqueous channels originated from pectin-chitosan, the higher amount of drug could release in F_1 and F_2 as compared to F_5 and F_6. These declarations can explain why there is no burst effect in the F_5 and F_6.

The interpretation of the results is not a straightforward process as there are two variables involved (coating weight gain and amount of pectin-chitosan) that interfere with the release process. However, in the majority of mentioned formulations, at the beginning of drug release, swelling or relaxation (case-II or super case-II transport) was the limiting step. Later, as the drug was depleted suddenly (burst effect), diffusion was the controlling mechanism.

Figure 3 illustrated the drug release profiles from enteric-coated capsules of F_9–F_{12} formulations. F_9–F_{12} formulations contain 20% w/w of theoretical coating weight gain and different amounts of pectin-chitosan (5, 10, 15, and 20% w/w, related to the total amount of mixed-film for F_9–F_{12}, respectively). The dissolution profiles of F_9 and F_{10} formulations were characterized by an initial lag phase corresponding to almost nothing or very slow drug release at the period of 120–180 min and a second lag phase at 300–360 min. These lag phases might be attributed to low amount of pectin-chitosan and the time required for the aqueous media to diffuse and stabilize the hydrodynamic exchanges across the mixed-film. After the lag phases, drug releases from these formulations were almost linear as a function of time. Unexpectedly, the drug release from F_9 and F_{10} showed a plateau after phase II, which suggested that the driving force responsible for drug release decreased

TABLE 4
Model Fitting of the Theophylline Release from F_5–F_8 Formulations

		F_5	F_6	F_7	F_8
Zero-order (phase II)	K_0 (mg/min)	0.1525	0.287	0.3644	0.4425
	R^2	**0.9735**	**0.9878**	**0.9946**	**0.9979**
Zero-order (phase III)	K_0 (mg/min)	0.1741	0.246	0.2397	0.2535
	R^2	0.9542	0.9818	**0.9962**	**0.9966**
First-order (phase II)	K_1 (1/min)	0.0214	0.0237	0.0129	0.0127
	R^2	0.9111	0.852	0.9413	0.9268
First-order (phase III)	K_1 (1/min)	0.0035	0.003	0.0021	0.0021
	R^2	**0.9879**	**0.9937**	0.9814	0.9822
Higuchi (phase II)	K_H (mg/min$^{1/2}$)	2.841	5.3394	6.8286	8.3406
	R^2	0.9299	0.9417	0.9618	0.9761
Higuchi (phase III)	K_H (mg/min$^{1/2}$)	6.1316	8.6982	8.5618	9.0566
	R^2	0.9295	0.9637	**0.998**	**0.9989**
Korsmeyer-Peppas (phase II)	K_{KP} (1/min^n)	0.0018	0.003	0.001	0.0012
	n	1.8632	2.1407	1.1075	1.1088
	R^2	0.9792	0.9859	0.9952	0.9984
Korsmeyer-Peppas (phase III)	K_{KP} (1/min^n)	0.0005	0.002	0.0117	0.0184
	n	1.0644	0.9235	0.6741	0.6195
	R^2	0.9694	0.98	0.9984	0.999

Amount of drug release and time expressed in mg and min, respectively; R^2 is the coefficient of determination.
Better results of R^2 are in bold.
K_0, K_1, K_H and K_{KP} are the release rates calculated from zero-order, first-order, and Higuchi and Korsmeyer-Peppas models, respectively.

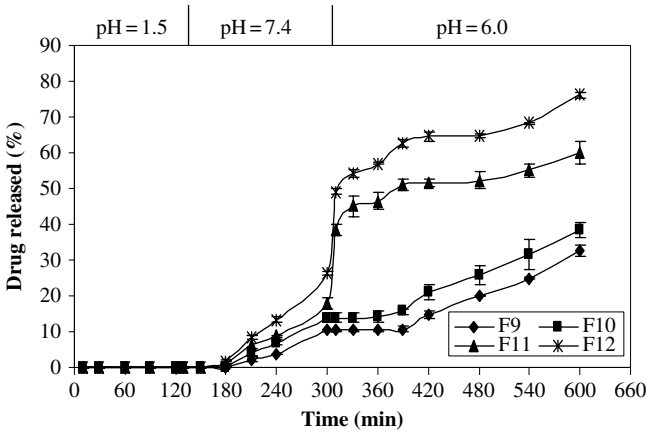

FIGURE 3. Cumulative percent of drug release versus time profiles for enteric-coated F_9–F_{12} formulations. The vertical lines on the upper x-axis denote the time points for the changes in dissolution media (Mean ± *SD*, $n = 3$).

dramatically. Moreover, Figure 3 shows that F_{11} and F_{12} formulations contain one lag phase (120–150 min) but in the colonic medium, their drug release profiles showed a burst release pattern. For F_9 and F_{10} formulations, during period of 210–300 min (phase II), n values were determined to be equal to 2.2728 and 1.7592, respectively (Table 5). During 180–300 min of phase II for the F_{11} and F_{12} formulations, n values were equal to 2.7328 and 2.5102, respectively. The values point to super case-II transport for those formulations. In addition, zero-order kinetics yielded best quality adjustment. Between 360 and 600 min for F_9 and 330–600 min for F_{10} (phase III), n values were resulted to be equal to 1.7394 and 1.3314, respectively. Moreover, between 310–600 min for F_{11} and F_{12}, n values were equal to 0.5389 and 0.6076, respectively. Values of n for F_9 and F_{10} formulations indicated that super case-II transport and zero-order kinetics was more correlated for both of them. In F_{11} and F_{12} formulations, n values pointing to anomalous transport and Higuchi model characterized better quality adjustments.

As can be seen in Figure 3, there were one and two lag phases in the release profiles of F_{11}–F_{12} and F_9–F_{10}, respectively. As a rule, the foregoing fitting analysis should be applied for the steady state in the drug release (Narasimhan, 2000). Drug delivery system does not comprise steady state condition in lag and burst release phases. Consequently, we believed that obtained n values from model fitting on F_9–F_{12} were not valuable because data points used for estimation of n were inadequate.

Similar points mentioned for F_5–F_6 could be repeated for F_9 and F_{10} formulations. The inadequate amount of pectin-chitosan against high coating weight gain could be responsible for the lag phases.

TABLE 5
Model Fitting of the Theophylline Release from F_9–F_{12} Formulations

		F_9	F_{10}	F_{11}	F_{12}
Zero-order (phase II)	K_0 (mg/min)	0.1872	0.219	0.2761	0.4091
	R^2	**0.9997**	**0.9992**	**0.9916**	**0.9971**
Zero-order (phase III)	K_0 (mg/min)	0.1894	0.1909	0.1181	0.1564
	R^2	**0.9858**	**0.9821**	0.8688	0.8901
First-order (phase II)	K_1 (1/min)	0.0173	0.0134	0.023	0.0214
	R^2	0.9703	0.982	0.7844	0.8274
First-order (phase III)	K_1 (1/min)	0.0051	0.0042	0.0012	0.0012
	R^2	0.9793	0.976	0.8243	0.8838
Higuchi (phase II)	K_H (mg/min$^{1/2}$)	4.2822	5.9561	5.8783	8.7019
	R^2	0.9531	0.9866	0.9867	0.895
Higuchi (phase III)	K_H (mg/min$^{1/2}$)	7.053	6.918	4.2718	5.6286
	R^2	0.9759	0.9691	**0.9920**	**0.9953**
Korsmeyer-Peppas (phase II)	K_{KP} (1/min^n)	0.002	0.0018	0.0033	0.0036
	n	2.2728	1.7592	2.7328	2.5102
	R^2	0.988	0.9983	0.8971	0.9297
Korsmeyer-Peppas (phase III)	K_{KP} (1/min^n)	0.0011	0.0001	0.0556	0.0627
	n	1.7394	1.3314	0.5389	0.6076
	R^2	0.9829	0.9706	0.8829	0.9271

Amount of drug release and time expressed in mg and min, respectively; R^2 is the coefficient of determination.
Better results of R^2 are in bold.
K_0, K_1, K_H and K_{KP} are the release rates calculated from zero-order, first-order, and Higuchi and Korsmeyer-Peppas models, respectively.

Possible Rationale(s) for the Burst Release

Pectin and chitosan belong to polysaccharides. Pectin-chitosan complex can be classified as a polyelectrolyte complex (PEC) hydrogel and this hydrogel is a type of physical hydrogels. Generally, hydrogels are polymer networks, which swell in water without dissolving. Hydrogels are usually made of hydrophilic polymers, which are crosslinked by various interactions such as chemical bonding, hydrogen bonding, ionic interactions, and hydrophobic interaction. Hydrogels are categorized into chemical and physical gels based on the nature of crosslinking. Physical hydrogels are formed by physical interactions between polymer chains. These interactions include hydrogen bonding, hydrophobic interactions, and ionic interactions. Ionic interactions between two oppositely charged polyelectrolytes (i.e., negatively charged pectin [P-COO^-] and positively charged chitosan [C-NH_3^+]) lead to the formation of PEC. Degradation of pectin and chitosan hydrogels occurs mostly through hydrolysis and enzymatic reaction (Chen, Jo, & Park, 1997).

There is still no agreement on a single definition for erosion and degradation of polymers. Therefore, it is necessary to define how these terms will be used. Degradation is the process of polymer chain scission by the cleavage of bonds between the monomers in the polymer backbone. Accordingly, degradation leads to a size reduction of the polymer chains. Erosion, in contrast, designates the breakdown of a polymer in a broader sense. Erosion is the mass loss of a polymer matrix that can result in loss of monomers, oligomers, or even pieces of non-degraded polymer. Erosion can be the result of biological, chemical, or physical effects. From the above definitions, it is obvious that polymer degradation is part of polymer erosion. In the biomedical field, biodegradation and bioerosion are significant (Göpferich, 1996).

There are four major modes of polymer degradation: photo-, mechanical-, thermal-, and chemical degradation. Of all degradation mechanisms, chemical degradation is the most important one for biodegradable polymers. By introducing hydrolysable functional groups into the polymer backbone, the polymer chains become labile to an aqueous environment and thus, chemical degradation initiates polymer erosion (Göpferich, 1997). In the current research, Pectinex® Ultra SP-L was used as pectinolytic enzymes. This is a highly active pectinolytic preparation produced by a selected strain of *Aspergillus Niger* and contains pectinolytic and a range of hemicellulolytic enzymes. Pectinex® is capable of hydrolytic break down of pectin substrate (Jayani, Saxena, & Gupta, 2005). The erosion of hydrolytically degradable polymers starts with the diffusion of water into the polymer. As the polymer chains are hydrated, the functional groups hydrolyze and absorb part of the water. During degradation, the polymer breaks down into oligomers and monomers. These compounds are transported from the polymer bulk. The release of degradation products leads to the mass

loss, which is a characteristic of erosion. Degradation is the most important part of erosion (Göpferich & Langer, 1995; Göpferich, 1997). The swelling and degradation of pectin-chitosan/Eudragit® RS mixed-film are depicted schematically in Figure 4.

The above explanation may help in better understanding the reason for burst release. During the phase two of drug release profile, the aqueous medium penetrates through the pellets' coating and starts dissolving the drug. It is assumed that the drug is immediately dissolved at the interface between solid and liquid. A semiliquid is formed inside the core and it coexists with the remaining solid core. The semiliquid part of the core becomes rich with the drug molecules and its concentration gradient gets higher than the external environment. Consequently, due to the positive gradient, the drug molecule is transported from the inside core to the outside. A diffusive release phenomenon is, therefore originated. A schematic representation of this phenomenon is shown in Figure 5. Possible reasons for the burst drug release at the beginning of third phase are discussed below lines. The enzymatic degradation occurred on the mixed-film, especially in loosen and microcrack of the surface. As mentioned previously, mixed-film exposure with the pectinolytic enzymes may result in degradation of pectin, and consequently partial erosion of film. We have mentioned that at the end of phase two, the drug molecules have already diffused and trapped within the whole coating volume and the mesh space of the coating network. After the coating exposure to pectinolytic enzymes, pectin is degraded and washed out from the film and, therefore, a sudden (burst) drug release may be observed. In other words, at the end of phase II, the drug molecules saturate the entire voids and pores in the coating and once in contact with enzyme, due to removal of pectin from the film, the porosity of film increases and the drug that has been diffused to the surface of the film is released immediately. There was a relative correlation between the percent of burst release and amount of pectin-chitosan complex in each set of formulations (F_1–F_4, F_5–F_8 and F_9–F_{12}), that is, the higher amounts of pectin-chitosan, the more burst released.

After burst effect, subsequent drug release is then caused by depletion and release from inside of the core. Moreover, drug release from degradable polymers also depends strongly on the structure of the eroding polymer matrix. Erosion zones and layers of monomers deposited on the surface of eroding polymer, act as diffusion barriers to released drugs (Göpferich, 1997). In addition, the enzymatic degradation and removal of pectin from the film can modify and suppress the hydrated pectin-chitosan channels (Semdé et al., 2000a). These phenomena lead to a decrease in the drug release rate after burst effect.

The above observation can be further justified by our proposed model. The current controlled release system can be considered a biodegradable reservoir system. We assumed that drug contains two pools inside coated pellets: a pool of mobile drug within and beneath the film that is free to diffuse upon hydration of the mixed-film, and a pool of immobilized drug loaded inside pellet core (Figure 5). The load of mobile drugs is depleted during short burst release. In other words, burst effect is associated with releasing of drug molecules in the mobile pool and film's void spaces.

Heller and Baker (1980) developed a model for erodible polymers. This model accounts for the effect of drug permeability in the biodegradable polymer, which is not constant, but increases with time, as more pores are created during the erosion. The following equation was used to account for the change of permeability with time as a function of number of remaining bonds. They assumed the below relationship between the drug permeability at time t, P_t, and the initial drug permeability, P_0:

$$P_t/P_0 = \text{number of initial bonds/number of remaining bonds} = N/N - Z \quad (5)$$

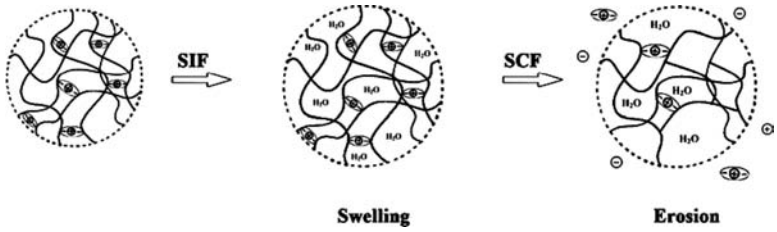

FIGURE 4. Schematic diagram of swelling/erosion of mixed-film of pectin-chitosan/Eudragit® RS; − Eudragit® RS; ionic interaction; pectin; + chitosan; SIF and SCF mean simulated intestinal fluid and simulated colonic fluid, respectively.

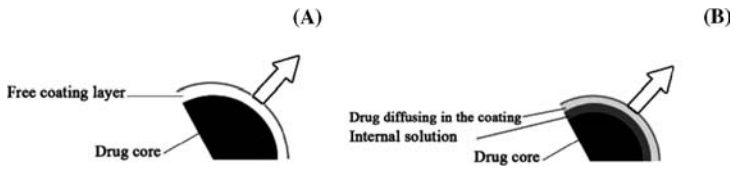

FIGURE 5. Schematic representations of drug releasing phenomenon for microencapsulated pellet, (A) intact pellet and (B) during drug release.

where N is the number of initial bonds, and Z is the number of bonds that have undergone cleavage during the time interval [0, t]. According to Equation 5, and after exposure of pectin-chitosan/Eudragit® RS to Pectinex®, the drug permeability through the mixed-film enhances, and it might be attributed to a decrease in the number of remaining bonds and, subsequently, an increase in the film's porosity (Figure 4).

Influence of the Pectinolytic Enzymes on the Drug Release from Coated Pellets

For this study, F_{12} was selected as an optimal formulation in viewpoint of high burst drug release and typical bimodal release characteristic. Figure 6 shows that the presence of the pectinolytic enzymes in the colonic dissolution medium resulted in an increase of the drug release from the coated pellets. A significant difference ($p < 0.001$) was observed in the percentage of drug release for presence and absence of the pectinolytic enzymes. Kinetics investigation verified that super case-II/zero-order transport was the prevailing mechanism at the second phase of release profile (data shown in Table 5). At pH 6.0, there were Higuchi and zero-order/Higuchi transport in the presence and absence of pectinolytic enzymes, respectively (Table 6). On the other hand, the existence of pectinolytic enzymes at phase III could cause a burst drug release; however, there was no difference between the two cases in slope of drug release after burst effect.

In general, the drug release from this coated system could be determined by an interplay of the following processes: (a) hydration and relaxation of the pectin-chitosan, (b) penetration of the dissolution medium towards the film, (c) solubilization/mobilization of drug, (d) diffusion of the mobile drug molecules from the bulk of pellet to its surface, (e) diffusion across the boundary layer, and (f) increasing the film's permeability due to pectin degradation.

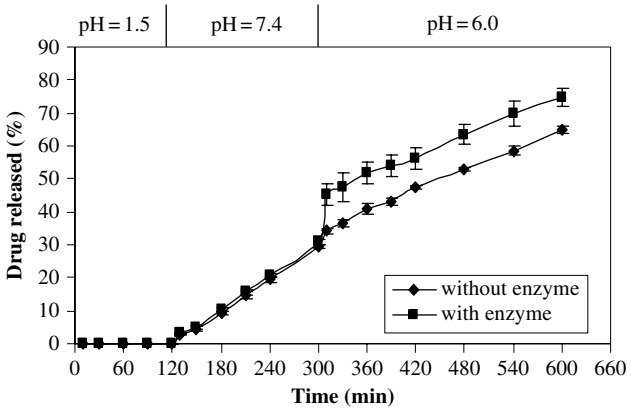

FIGURE 6. Cumulative percent of drug release versus time profile for enteric-coated F_{12} formulation, in the presence or absence of pectinolytic enzymes in simulated colonic medium (phosphate buffer pH 6.0). The vertical lines on the upper x-axis denote the time points for the changes in dissolution (Mean ± SD, $n = 3$).

TABLE 6
Model Fitting of the Theophylline Release from F_{12} Formulation in Two Cases: With and Without Pectinolytic Enzymes in the Simulated Colonic Medium

		With Enzyme	Without Enzyme
Zero-order	K_0 (mg/min)	0.2030	0.2080
(phase III)	R^2	0.8954	**0.9963**
First-order	K_1 (1/min)	0.0016	0.0021
(phase III)	R^2	0.9881	0.9804
Higuchi	K_H (mg/min $^{1/2}$)	7.2429	7.4407
(phase III)	R^2	**0.9962**	**0.9973**
Korsmeyer-Peppas	K_{KP} (1/min^n)	0.0266	0.0096
(phase III)	n	0.5395	0.6816
	R^2	0.9962	0.9976

Amount of drug release and time expressed in mg and min, respectively; R^2 is the coefficient of determination.

Better results of R^2 are in bold.

K_0, K_1, K_H and K_{KP} are the release rates calculated from zero-order, first-order, and Higuchi and Korsmeyer-Peppas models, respectively.

CONCLUSION

The present study was carried out to receive detailed information on the drug release from microcapsule-based pectin-chitosan/Eudragit® RS. The mechanism of drug release from each formulation was evaluated in the terms of zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. The drug release from our fabricated system did not follow a single mechanism, but it would seem that several mechanisms were competing at the same time. Zero-order kinetics was found as the better fitting model for all formulations at phase II drug release (small intestine). At third phase (colonic medium) for the majority of formulations, an anomalous transport was obtained and both zero-order and Higuchi model characterized better adjustment.

It would seem that pectin, chitosan and Eudragit® RS produced a heterogeneous mixed-film structure. In an aqueous environment, akin to the conditions of the dissolution test or the gastrointestinal tract, pectin-chitosan swelled and disrupted the organization of the mixed-film, leading to the formation of aqueous filled channels (pores). These channels provide pathways for drug diffusion from the core of the pellet to the external medium. This route of release is in addition to drug diffusion through the Eudragit® RS component of the film. It was observed that the rate and pattern of drug release could be controlled by amount of pectin-chitosan incorporated in mixed-film and by changing the coating weight gain.

We have mentioned that at the end of phase two, some of drug molecules, have already diffused and have been trapped within the coating volume and the mesh space of the film network. After the film exposure to pectinolytic enzymes, pectin is degraded and removed from the coating and therefore a sudden (burst) release occurrs. In other words, due to degradation and removal of pectin from the film, the porosity of film increases and the drug molecules that have already been diffused to the surface release immediately. Generally, pore formers can increase the drug release (Frohoff-Hülsmann, Schmitz, & Lippold, 1999). According to Iyer et al. (1990), a higher porosity leads to an increase in the diffusion coefficient, which results in a higher drug permeability and a faster release rate. In the current investigation, the slope of drug release declines after burst effect. This may be rationalized by the following discussion. After the enzymatic breakdown and leaching of pectin, the mixed-film can restructure, plug up, and reduce the free volume between polymer chains and, therefore, slow down the drug release. In addition, the removal of pectin can modify/suppress the hydrated pectin-chitosan channels.

By using a balanced ratio between coating weight gain and pectin-chitosan amount, a suitable bimodal release can be achieved. A substantial amount of pectin-chitosan incorporated in the mixed-film can rapidly absorb the surrounding water. The inadequate amount of pectin-chitosan may be responsible for no burst release or lag phase. At high coating weight gains (i.e., 15 or 20% w/w) and insufficient amount of pectin-chitosan (i.e., 5 or 10% w/w), film barrier predominately governs drug release because all pectin-chitosan aqueous channels are blocked. Therefore, the drug must take the pathway involving diffusion through the barrier; this can be related to drug permeability in the film and the film thickness.

The main thrust of this work is that the release of drug molecules occurs on two distinct time scales: The partial depletion of the pool of mobile drug is controlled by film swelling (relaxation) in small intestine medium, whereas the burst release (complete depletion of that pool) is predominantly controlled by polymeric degradation process in the colonic medium. Indeed, this enzymatically degradable system exhibits burst release effect followed by an anomalous release in the colonic environment.

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