AN ABSTRACT OF THE DISSERTATION OF


Title: 1) Pharmacokinetic Modeling and Simulations of Gastrointestinal Transit Effects on Drug Pharmacokinetics from Enteric-Coated Pellet Formulations and Their Applications 2) Development of Crushable Enteric-Coated Formulations 3) Development of Leaky Enteric-Coated Pellets Formulations

Abstract approved: Redacted for privacy

James W. Ayres

Effects of gastrointestinal transit on plasma concentrations of drugs from enteric-coated pellet formulations were demonstrated using pharmacokinetic models describing plasma concentrations of drugs from various enteric-coated pellet formulations. Gastric emptying time, lag time of emptying, and drug release rate from pellets in the small intestine, along with other pharmacokinetic parameters of drugs, were used to construct pharmacokinetic models. The models were then evaluated by comparing simulated plasma concentrations of model drugs from Monte Carlo simulations to observed plasma concentrations of these drugs from the literature. Results showed that the models described plasma concentrations of drugs from enteric-coated pellet formulations very well. Pharmacokinetic models describing plasma concentrations of drug from mixed immediate-release and enteric-coated pellet formulations were also used in simulations of bioequivalence.
studies. Results from the research are very useful in designing generic products of mixed pellet formulation and in refining or selecting the final product for actual bioequivalence study.

Development of crushable enteric-coated formulations was presented. Nonpareil sugar pellets were spray-loaded with mixed amphetamine salts. Drug-loaded pellets were subsequently spray-coated with enteric polymer, hydrophilic gel-forming polymer, enteric polymer and/or mixture of insoluble polymer and hydrophilic polymer. The resulting pellets were then spray-coated with disintegrant and compressed to form crushable tablets. Dissolution testing of both non-compacted crushable enteric-coated tablets and crushed tablets showed that the intact crushable tablet formulations and the crushed tablet formulations were able to prevent the majority of the drug from being released in a simulated gastric dissolution medium within first 2 hours.

Concept and formulations of “leaky” enteric-coated pellets were presented. “Leaky enteric-coated pellets” formulation is defined as enteric-coated pellets that allow some of the drug to be released from the formulation in acidic dissolution medium. Different approaches of making leaky enteric-coated pellets using spray-coating techniques were presented. Plasma concentrations of drug from leaky enteric-coated pellet formulations were simulated using computer simulations. The present research was based on the hypothesis that leaky enteric-coated pellets formulations were able to provide sustained-release effect on plasma concentration profiles of drugs that have the absorption window without jeopardizing their bioavailability or with improved bioavailability.
1) Pharmacokinetic Modeling and Simulations of Gastrointestinal Transit Effects on Drug Pharmacokinetics from Enteric-Coated Pellet Formulations and Their Applications
2) Development of Crushable Enteric-Coated Formulations
3) Development of Leaky Enteric-Coated Pellets Formulations

by
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Major Professor, representing Pharmacy

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Dean of the College of Pharmacy

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Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Prapoch Watanalumlerd, Author
I would like to express my sincere appreciation to several people who have involved in my study at Oregon State University. First of all, I would like to thank Dr. James W. Ayres, my major advisor, whom I had a privilege working directly with during past five years. I am grateful for his guidance, support, and his commitment to my learning. His kindness and understanding is much appreciated. I also thank Dr. J. Mark Christensen and Dr. Rosita R. Proteau for their guidance and best wishes throughout the years. Their contributions to my learning at the College of Pharmacy are invaluable.

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DEDICATION

To David
CHAPTER 1

GENERAL INTRODUCTION

Enteric coating has been widely used in dosage form design. There are several intended uses of enteric coating including drug stabilization, protection against local irritation, and regional drug delivery within the digestive tract (1). When administered as an enteric-coated formulation, drug is protected from gastric acidity and is not released until the dosage form reaches the intestine (1). Consequently, patterns of drug absorption of enteric-coated dosage forms differ from those of immediate-release dosage forms. A delay or lag time of absorption is expected from enteric-coated formulation due to a delay of drug release in the gastrointestinal (GI) tract (2). Patterns of drug absorption from enteric-coated formulations are known to be influenced by physiological factors and formulation characteristics (1), for example, the GI transit time, effects of food, pH of the GI tract, types of polymer and dosage form.

When multiple-unit enteric-coated pellet formulation is administered, the presence of drug in plasma or blood will be influenced by the aforementioned factors. Plasma concentrations will be different from those of single-unit enteric-
coated formulation and those of immediate release formulation. Prediction of plasma concentrations of drug following an administration of enteric-coated pellets, therefore requires understanding of physiological factors and characteristics of enteric-coated formulation.

In Chapters 2 and 3, effects of GI transit on plasma concentrations of drugs from enteric-coated pellet formulations are demonstrated. Several pharmacokinetic models describing plasma concentrations of drugs from various enteric-coated pellet formulations were developed. Gastric emptying time, lag time of emptying, and drug release rate from pellets in the small intestine, along with other pharmacokinetic parameters of drugs, were used to construct pharmacokinetic models. The models were then evaluated by comparing simulated plasma concentrations of model drugs from Monte Carlo simulations to observed plasma concentrations of these drugs from the literature. Amphetamine and erythromycin were used as model drugs because of availability of published literature data.

Chapter 4 elaborates another application of pharmacokinetic models developed in Chapters 2 and 3. Pharmacokinetic models describing plasma concentrations of drug from mixed immediate-release and enteric-coated pellet formulations were used in simulations of expected bioequivalence study results. Effects of drug release rate from enteric-coated pellets in the intestine and its' variability on probability of passing bioequivalence studies were considered.

Chapter 5 presents crushable enteric-coated pellet formulations. Nonpareil sugar pellets were spray-loaded with a model drug, mixed amphetamine salts. Drug-loaded pellets were subsequently spray-coated with enteric polymer, hydrophilic gel-forming polymer, enteric polymer and/or mixture of insoluble polymer and hydrophilic polymer. The resulting pellets were then spray-coated with disintegrant and compressed to form crushable tablets. Dissolution testing of non-crushed tablets and crushed tablets showed that both intact crushable tablet
formulations and the crushed tablet formulations were able to prevent the majority of drug from being released into simulated gastric dissolution medium in 2 hours.

In Chapter 6, concepts and formulations of “leaky” enteric-coated pellets are presented. “Leaky enteric-coated pellet” formulation is defined as enteric-coated pellets that allow some of the drug to be released from the formulation in acidic dissolution medium. Riboflavin-5-phosphate, ranitidine hydrochloride, and hydrochlorothiazide were used as model drugs. Different approaches of making leaky enteric-coated pellets using spray-coating techniques are presented. Plasma concentrations of drug from leaky enteric-coated pellet formulations were simulated using computer simulations. Effects of varying leakage rate of pellets in the stomach on predicted plasma concentration profiles are shown.
REFERENCES


CHAPTER 2

PHARMACOKINETIC MODELING AND SIMULATIONS OF GASTROINTESTINAL TRANSIT EFFECTS ON PLASMA CONCENTRATIONS OF DRUGS FROM MIXED IMMEDIATE-RELEASE AND ENTERIC-COATED PELLET FORMULATIONS—INSTANTANEOUS DRUG RELEASE MODELS

Prapoch Watanalumlerd, J. Mark Christensen, and James W. Ayres
ABSTRACT

Effects of gastrointestinal transit on plasma concentrations of drugs from mixed immediate-release and enteric-coated pellet formulation were simulated with models developed by including gastric emptying time and lag time of emptying, along with other pharmacokinetic parameters of drugs. Models were then evaluated by comparing simulated plasma concentrations of amphetamine from Monte Carlo simulations to observed plasma concentrations of amphetamine from available published data of a commercial mixed immediate-release and enteric-coated pellet formulation (Adderall XR™). Results show that the plasma drug concentration-time curve from the mixed pellet formulation does not mimic that from two immediate-release doses administered at different times. Instead, the plasma drug concentration-time curve from the mixed pellets is similar to a typical sustained-release formulation. The pharmacokinetic models presented herein predict plasma concentrations of amphetamine from mixed immediate-release and enteric-coated pellet formulation quite well. The models and assumptions are general and can be applied to other drugs.
INTRODUCTION

Patterns of drug absorption from enteric-coated formulations are known to be influenced by physiological factors and formulation characteristics (1). A delay of absorption can be expected from enteric-coated formulations due to a delay of drug release in the gastrointestinal (GI) tract (2). Formulations of different size may have different transit time through the GI tract. Drug pellets (0.7-1.2 mm in diameter) were shown to have shorter transit time through the stomach than a single-unit formulation of larger size in the presence of food (an osmotic pump, Osmet 12-hour system) (3). Multiple-unit enteric-coated pellets were demonstrated to produce less intrasubject and intersubject variation in absorption than single-unit enteric-coated tablets upon immediate administration after breakfast (4).

GI transit time of drug pellets has been extensively studied. Food was shown to have a profound effect on gastric emptying rate of drug pellets (3, 5, 6). In the fed condition, gastric emptying rate of pellets appears to be zero order over 5 to 8 hours (6-8). These findings are consistent with another study that found emptying of solids is approximately a zero-order function (9). Meal size has been shown to have an influence on half-time of gastric emptying of pellets. The mean half-time was 78 minutes after a light meal (1500 kJ or 358.5 kcal) compared to 170 minutes for a heavy meal (3600 kJ or 860.4 kcal) (5). In the fasted condition, fifty percent of pellets are emptied from the stomach within an hour with a range of less than 0.3 to 0.9 hour (6) depending upon the time of administration relative to an occurrence of phase 3 of the migrating myoelectric complex (MMC) (10). It is known that emptying of non-nutrient-containing liquid appears to be first order and volume-sensitive mechanisms play the major role in the regulation of gastric emptying (10). Patterns of gastric emptying of pellets taken before a meal were
shown to be approximately exponential, i.e., typical of gastric emptying of liquid (11).

Lag time of gastric emptying for solid food also differs from that for liquid. The initial lag phase has been observed for gastric emptying of solid food and the average values range from 21 to 60 minutes (6, 9, 12). This lag time reflects primarily the time required to reduce the solid food to smaller sizes (13). After a capsule containing drug pellets was administered in the fed condition, seven of eight subjects showed no gastric emptying of the pellets during the first hour (6). This observed delay of the emptying of pellets suggests that, following capsule disintegration, the pellets became dispersed within the stomach and were mixed with food content before being emptied along with the meal (11). Lag time of gastric emptying in these subjects causes lag time of absorption for drug in enteric-coated pellets (6). Unlike solid food, emptying of liquid has minimal observable lag time (9).

While presence of food increases the mean gastric emptying time of pellets, the small intestinal transit time is unaffected (3). The mean small intestinal transit time is about 3 to 4 hours (3, 5, 7, 14) and independent of the feeding state (3, 5). Multiple-unit pellets and non-disintegrating single-unit tablets have similar small intestinal transit time (3). Depending on the feeding state, the mean time for the arrival at the caecum of pellets ranges from 4 to 8 hours (3, 5, 7, 14).

A commercial drug product formulation containing mixed immediate-release and enteric-coated pellets of mixed amphetamine salts (Adderall XR™) has been developed and purported to give a double-pulsed delivery mimicking administration of two immediate-release doses at different times (15). However, the published plasma concentration of amphetamines from this formulation did not show a pattern of double-pulsed delivery. Rather, the plasma concentration of amphetamines (Figure 2.1) was similar to a typical sustained-release formulation.
Unlike the mixed immediate-release and enteric-coated pellets formulation of amphetamines salts, the plasma concentration of melatonin (Figure 2.2) following simultaneous administration of immediate-release tablet and enteric-coated tablet (two different tablets in a capsule) clearly showed double-pulsed release of the drug from the formulation (16).

**Figure 2.1** Adderall XR™ and Adderall® plasma concentrations in fed subjects. Mean d-amphetamine and l-amphetamine plasma concentrations following administration of Adderall XR™ (mixed pellets) 20 mg and Adderall® (immediate release) 10 mg (two doses, four hours apart) in fed subjects (from reference (15) with permission).
Figure 2.2 Melatonin serum concentration-time profiles. Individual melatonin serum concentration-time profiles after oral administration of capsule containing an immediate-release tablet and an enteric-coated tablet (from reference (16) with permission).

The difference in plasma concentration patterns from the two different enteric-coated formulations (a capsule containing a mixture of immediate-release and enteric-coated pellets compared to a combined immediate-release and enteric-coated tablet) are shown to be expected based on physiological and drug formulation factors known to be significantly responsible for characteristics of drug release from the formulations, and subsequent drug absorption. That is, since enteric-coated tablets empty from the stomach as a single unit the double-pulsed release is expected, and because enteric-coated pellets “trickle” from the stomach, a more sustained pattern of release is expected, as observed.

Other than being described by the zero or the first-order process, gastric emptying has also been described using other mathematical equations/functions, e.g. a power exponential equation, (17) and a two-component linear function. (18) Based on simplicity and feasibility, zero-order emptying and first-order emptying
processes are implemented in the present work for the fed and fasted condition, respectively.

It is an objective of the present work to apply pharmacokinetic modeling to the fed and fasted human subjects using known GI transit parameters to predict plasma drug concentrations from mixed immediate-release and enteric-coated drug pellet formulations. Monte Carlo simulation is applied to the models to include the effect of GI transit variability on simulated plasma concentrations of drug from mixed immediate-release and enteric-coated pellets. Available pharmacokinetic data in the fed and fasted condition for the mixed amphetamines pellets are used to evaluate the models.
MATERIALS AND METHODS

Pharmacokinetic Models

Using knowledge about gastric emptying and GI transit, compartmental diagrams illustrating pharmacokinetics of drugs from mixed immediate-release and enteric-coated pellets in the fed and fasted condition are created and shown in Figures 2.3 and 2.4, respectively. Compartmental diagrams in Figure 2.3 represent (i) first-order absorption of immediate-release pellets and (ii) zero-order gastric emptying rate in the fed condition of enteric-coated pellets into the intestine, and first order absorption of the drug after being released from the pellets. Compartmental diagrams in Figure 2.4 represent (i) first-order absorption of immediate-release pellets and (ii) first-order gastric emptying rate in the fasted condition of enteric-coated pellets into the intestine, and first order absorption of the drug after being released from the pellets. These compartmental diagrams apply to drugs when pharmacokinetics following oral administration can be described by a one-compartment model.
Immediate Release Pellets

\[
\begin{align*}
D_{IR} & \xrightarrow{k_a} X_1 \\
\text{Drug in} & \quad \text{Blood} \\
\text{GI tract} & \end{align*}
\]

Enteric-Coated Pellets

\[
\begin{align*}
D_{EC} & \xrightarrow{k_0} X_G \\
\text{Pellets in} & \quad \text{Drug in} \\
\text{the stomach} & \quad \text{the intestine} \\
\text{Drug in} & \quad \text{Blood} \\
\text{Blood} & \end{align*}
\]

Figure 2.3 Compartmental diagrams of pharmacokinetic models for mixed immediate-release and enteric-coated pellets in fed condition.

Immediate Release Pellets

\[
\begin{align*}
D_{IR} & \xrightarrow{k_a} X_1 \\
\text{Drug in} & \quad \text{Blood} \\
\text{GI tract} & \end{align*}
\]

Enteric-Coated Pellets

\[
\begin{align*}
D_{EC} & \xrightarrow{k_{em}} X_G \\
\text{Pellets in} & \quad \text{Drug in} \\
\text{the stomach} & \quad \text{the intestine} \\
\text{Drug in} & \quad \text{Blood} \\
\text{Blood} & \end{align*}
\]

Figure 2.4 Compartmental diagrams of pharmacokinetic model for mixed immediate-release and enteric-coated pellets in fasted condition.

Notation: \(X_G\), amount of released drug in the intestine; \(X_1\), amount of drug in blood; \(D_{IR}\), an immediate-release dose; \(D_{EC}\), an enteric-coated dose; \(k_0\), a zero-order input of drug corresponding to a zero-order gastric emptying of enteric-coated pellets in fed condition; \(k_{em}\), a first-order rate of drug input corresponding to a first-order gastric emptying of enteric-coated pellets in fasted condition; \(k_a\), a first-order absorption rate constant; \(k_{el}\), a first-order elimination rate constant.
Pharmacokinetic models, which describe plasma concentrations of a drug following oral administration of controlled release dosage forms exhibiting zero-order and first-order release kinetics, have been previously developed (19, 20). By combining the pharmacokinetic models of oral controlled zero-order release dosage form (19) with a typical extravascular pharmacokinetic model (for immediate-release pellets), models describing pharmacokinetics of mixed immediate-release and enteric-coated pellets in the fed condition are now obtained and presented in Equations 2.1 and 2.2.

For fed condition

When $t \leq \tau$,

$$C_t = \frac{k_a D_{IR}}{V(k_a - k_{el})} \left( e^{-k_{el}t} - e^{-k_a t} \right)$$

$$+ \frac{k_0}{V \cdot k_{el}} \left[ 1 - \frac{k_{el}}{(k_{el} - k_a)} e^{-k_a t} - \frac{k_a}{(k_a - k_{el})} e^{-k_{el} t} \right]$$

When $t > \tau$,

$$C_t = \frac{k_a D_{IR}}{V(k_a - k_{el})} \left( e^{-k_{el}t} - e^{-k_a t} \right)$$

$$+ \frac{k_0}{V \cdot k_{el}} \left[ 1 - \frac{k_{el}}{(k_{el} - k_a)} e^{-k_a \tau} - \frac{k_a}{(k_a - k_{el})} e^{-k_{el} \tau} \right] e^{-k_{el}(t-\tau)}$$

$$+ \frac{k_0}{V(k_a - k_{el})} \left[ 1 - e^{-k_{el} \tau} \right] e^{-k_{el}(t-\tau)} - e^{-k_a(t-\tau)}$$

For the fasted condition, a pharmacokinetic model describing plasma concentration of a drug from mixed immediate-release and enteric-coated pellets
can be obtained by combining the pharmacokinetic model of oral controlled first order release dosage form (20) with a typical extravascular pharmacokinetic model for immediate-release pellets. This model is presented in Equation 2.3.

For fasted condition

\[
C_t = \frac{k_a D_{IR}}{V(k_a - k_{cl})} (e^{-k_{at}t} - e^{-k_{dt}t}) + \frac{k_{em}k_a D_{EC}}{V} e^{-k_{em}t} \left[ \frac{1}{k_a - k_{em}} \frac{1}{k_{em} - k_{cl}} \left( e^{-k_{em}t} - e^{-k_{cl}t} \right) \right]
\]

where \( C_t \) is plasma concentration of the drug at time \( t \). \( D_{IR} \) is an immediate-release dose. \( D_{EC} \) is an enteric-coated dose. \( k_{em} \) represents a first-order rate of drug input corresponding to a first-order gastric emptying of enteric-coated pellets in fasted state. \( k_0 \) represents a zero-order input of drug corresponding to a zero-order gastric emptying of enteric-coated pellets in fed state. \( k_a \) and \( k_{cl} \) represent a first-order absorption rate constant and a first-order elimination rate constant of drug, respectively. \( \tau \) is gastric emptying time of enteric-coated pellets (i.e. the time of zero-order input). \( V \) is an apparent volume of distribution for the blood compartment. These equations may be multiplied by a bioavailability factor \( F \), which is the fraction of absorbed drug.

Since lag time of gastric emptying is expected and will affect drug release from enteric-coated pellets, the above equations are modified by including another time parameter—lag time of emptying (\( lag \)), presented in Equations 2.4 to 2.8.
For fed condition

When $t \leq \text{lag}$,

$$C_t = \frac{k_a D_{IR}}{V(k_a - k_{el})} \left( e^{-k_{el}t} - e^{-k_a t} \right)$$  \hspace{1cm} 2.4

When $\text{lag} < t \leq \tau + \text{lag}$,

$$C_t = \frac{k_a D_{IR}}{V(k_a - k_{el})} \left( e^{-k_{el}t} - e^{-k_a t} \right)$$

$$+ \frac{k_0}{V \cdot k_{el}} \left[ 1 - \frac{k_{el}}{k_a - k_{el}} e^{-k_a t} - \frac{k_a}{k_a - k_{el}} e^{-k_{el} t} \right]$$  \hspace{1cm} 2.5

When $t > \tau + \text{lag}$,

$$C_t = \frac{k_a D_{IR}}{V(k_a - k_{el})} \left( e^{-k_{el}t} - e^{-k_a t} \right)$$

$$+ \frac{k_0}{V \cdot k_{el}} \left[ 1 - \frac{k_{el}}{k_a - k_{el}} e^{-k_a \tau} - \frac{k_a}{k_a - k_{el}} e^{-k_{el} \tau} \right] e^{-k_{el} \tau}$$

$$+ \frac{k_0}{V(k_a - k_{el})} \left[ e^{-k_{el}(\tau + \text{lag} - t)} - e^{-k_a(\tau + \text{lag} - t)} \right]$$  \hspace{1cm} 2.6

For fasted condition

When $t \leq \text{lag}$,

$$C_t = \frac{k_a D_{IR}}{V(k_a - k_{el})} \left( e^{-k_{el}t} - e^{-k_a t} \right)$$  \hspace{1cm} 2.7
When $t > \text{lag}$,

$$C_t = \frac{k_a D_{IR}}{V (k_a - k_{cl})} \left( e^{-k_{el} t} - e^{-k_a t} \right)$$

$$+ \frac{k_{em} k_a D_{EC}}{V} \left[ \frac{e^{-k_{em}(t-\text{lag})}}{(k_a - k_{em})(k_{cl} - k_{em})} \right.$$  

$$\left. + \frac{e^{-k_a(t-\text{lag})}}{(k_{em} - k_a)(k_{cl} - k_a)} + \frac{e^{-k_{cl}(t-\text{lag})}}{(k_{em} - k_{cl})(k_a - k_{cl})} \right]$$

### Model Assumptions

Assumptions for these models are:

1) Pharmacokinetic of the drug is linear in the dosing range of interest. Thus, superposition for determination of plasma drug concentrations can be applied.

2) Enteric-coated portion of formulations is in multiple-unit pellet/granule (multi-particulate) form.

3) Enteric-coated polymer dissolves instantaneously upon transfer into the intestine.

4) After the pH-dependent polymer on enteric-coated pellets dissolves, release of the drug is instantaneous (i.e. similar to that of immediate-release dose).

5) Once being released from formulations, the drug is absorbed from the gastrointestinal tract by a first-order process.

6) Pharmacokinetic of the drug after absorption is well described by a one-compartment open model.

7) The elimination process is a first-order process.
Monte Carlo Simulations

Pharmacokinetic models above were used in Monte Carlo simulations of plasma concentration-time curves from mixed immediate release and enteric-coated pellets of amphetamine. Five hundred (500) trials for each simulation were performed using Crystal Ball 2000.2 software (Decisioneering, Inc., Denver, CO). The simulated plasma concentration-time curves of amphetamine are presented as a mean plot (along with its standard deviation) and as a percentile plot. The peak plasma concentration ($C_{\text{max}}$) of the actual data is then compared to $C_{\text{max}}$ of simulated data.

Model Parameters

Following oral administration of immediate-release amphetamine, a one-compartment model best describes plasma drug concentrations both in adults and children (21, 22). Pharmacokinetic parameters of amphetamine used in the simulations were obtained from pharmacokinetic fitting of available plasma concentration data of amphetamine (15) using Kinetica 2000 software, version 3.0 (InnaPhase Corporation, Philadelphia, PA). These parameters are volume of distribution (V/F), absorption rate constant ($k_a$), and elimination rate constant ($k_{el}$). Since pharmacokinetics of d-amphetamine and l-amphetamine are similar, only simulations of d-amphetamine will be carried out. Pharmacokinetic parameters of d-amphetamine used in the simulations are summarized in Table 2.1.

Parameters included in the models to represent GI transit effect are gastric emptying rate constant and lag time of gastric emptying. The gastric emptying rate constant is zero order for the fed condition and first order for the fasted condition.
Table 2.1 Pharmacokinetic Parameters of Model Drug Used in Simulations

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose $^a$ (mg)</th>
<th>V/F (L)</th>
<th>$k_a$ (hr$^{-1}$)</th>
<th>$k_el$ (hr$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Amphetamine</td>
<td>20 (fed)</td>
<td>247.0</td>
<td>0.744</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td>30 (fasted)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Dose represents mixed amphetamine salts dose. Twenty (20) milligrams of the mixed amphetamine salts is equivalent to 12.5 mg of total amphetamine base and contains d-amphetamine and l-amphetamine salts in the ratio of 3:1 (15). Pharmacokinetic parameters are assumed to be the same for different doses.

Variability of Model Parameters

In Monte Carlo simulations, variability of some or all model parameters is included in the simulations. Because effects of gastric emptying on the plasma concentration-time curve are being considered, variability of gastric emptying time and lag time of emptying was included in the simulations. Variability of other model parameters (e.g. $k_{el}$, $k_a$, V, F) was not included.

Gastric emptying time, lag time of emptying and their variability (standard deviation) were obtained from the literature (5, 6, 9, 14). A lognormal distribution was chosen for all time parameters since time cannot be negative. T$_{50}$ is utilized for calculation of a first-order emptying rate constant in the fasted condition. Lag time of emptying in the fasted condition was selected based on phase 1, a period of motor inactivity, of MMC, which lasts approximately 30 to 60 min. Variability for lag time of emptying in the fasted condition was assumed to be 30 percent. Model parameters and their probability distribution used in the simulations are detailed in Table 2.2.
Table 2.2 Probability Distribution of Model Parameters and Their Mean and Standard Deviation

<table>
<thead>
<tr>
<th>Parameters (unit)</th>
<th>Distribution</th>
<th>Mean ± SD (ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fed Condition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag time of emptying (hr)</td>
<td>Lognormal</td>
<td>1 ± 0.37 (9)</td>
</tr>
<tr>
<td>Gastric emptying time (hr)</td>
<td>Lognormal</td>
<td>5.7 ± 0.9 (5)</td>
</tr>
<tr>
<td><strong>Fasted Condition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag time of emptying (hr)</td>
<td>Lognormal</td>
<td>0.75 ± 0.22 (14)</td>
</tr>
<tr>
<td>$T_{50}$ (hr) $^a$</td>
<td>Lognormal</td>
<td>0.5 ± 0.2 (6)</td>
</tr>
</tbody>
</table>

$^a$ First-order emptying rate constant is calculated from $0.693/T_{50}$.

Determination of Amphetamine Absorption Profile from Mixed Immediate-release and Enteric-coated Pellets

Deconvolution of available plasma amphetamine concentration profiles (23) from commercial mixed immediate-release and enteric-coated pellets was performed using Kinetica 2000 software, version 3.0 (InnaPhase Corporation, Philadelphia, PA), to obtain the absorption profiles of d-amphetamines.

In Vitro Dissolution Profile of Mixed Immediate-release and Enteric-coated Pellets

Commercial mixed immediate-release and enteric-coated amphetamine capsules (Adderall XR$^{TM}$) containing 10 mg of immediate-release amphetamine salts and 10 mg of delayed release amphetamine salts were used for in vitro dissolution test. An amphetamine dissolution profile was obtained using USP
dissolution apparatus II at 37.5 °C and paddle rotating speed at 100 rpm. The formulation without capsule shell was run in simulated gastric fluid (pH 1.4) for 2 hours before the dissolution medium was adjusted to pH 6.0 by adding alkaline solution (0.2 M tribasic sodium phosphate solution) and de-ionized water. Samples were assayed for amphetamine concentration using an ultraviolet spectrophotometer at 257 nm.
RESULTS AND DISCUSSION

Absorption profiles of d-amphetamine from deconvolution of known plasma concentration profiles of commercial mixed immediate-release and enteric-coated pellets in fed (Figure 2.1) and fasted subjects (Figure 2.10) are presented in Figure 2.5. Absorption profile of d-amphetamine from deconvolution of two 10-mg doses of immediate-release formulation of amphetamine (Adderall®) in fed subjects was compared with d-amphetamine absorption profile from commercial mixed immediate-release and enteric-coated pellets in Figure 2.6.

![Graph](image)

**Figure 2.5** Absorption profiles of d-amphetamine from commercial mixed immediate-release and enteric-coated pellets in fed and fasted subjects.
Figure 2.6 Absorption profiles of d-amphetamine from 20 mg dose of Adderall™ XR (commercial mixed immediate-release and enteric-coated pellets) and from two 10-mg doses of immediate-release Adderall® (given four hours apart) in fed subjects.

The absorption profiles of d-amphetamine from the commercial mixed pellet formulation show no pattern of double-pulsed absorption as observed in two doses of immediate-release formulation, even though the formulation is promoted as a pulsed dose formulation. Instead, the absorption profiles resemble a typical sustained-release formulation with a short lag time of absorption in fed subjects.

An in vitro dissolution profile of the commercial mixed pellet formulation is shown in Figure 2.7. Amphetamine was completely released from immediate-release pellets in 15 minutes. After adjusting the dissolution medium to pH 6.0, the drug was completely released from enteric-coated pellets in about 30 minutes. Such
rapid release of the drug from both types of pellet justifies the assumption of the models about instantaneous drug release.

![Graph showing percentage drug release over time with pH values at 1.4 and 6.0.]

**Figure 2.7** In vitro dissolution profile of commercial mixed immediate-release and enteric-coated pellets of amphetamines.

**Table 2.3** Summary of the Predicted and Observed \( C_{\text{max}} \) of d-Amphetamine

<table>
<thead>
<tr>
<th>Condition (Dose)</th>
<th>Predicted ( C_{\text{max}} ) (ng/ml)</th>
<th>Observed ( C_{\text{max}} ) a (ng/ml)</th>
<th>%Difference b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted (30 mg)</td>
<td>43.6</td>
<td>40.2</td>
<td>8.4</td>
</tr>
<tr>
<td>Fed (20 mg)</td>
<td>26.0</td>
<td>25.3</td>
<td>2.8</td>
</tr>
</tbody>
</table>

a Observed fasted and fed data were obtained from reference (23).
b %Differences are presented as percentage of the observed \( C_{\text{max}} \).
Average predicted peak concentrations ($C_{\text{max}}$) from the simulations and observed $C_{\text{max}}$ in fed and fasted subjects are presented in Table 2.3. A mean plot and a percentile plot of simulated plasma concentration-time curve of amphetamine from mixed immediate-release and enteric-coated pellets in the fed condition are presented in Figures 2.8 and 2.9, respectively.

Figure 2.8 Mean plot of simulated plasma concentrations of d-amphetamine from mixed immediate-release and enteric-coated pellets in fed subjects. Vertical bars represent standard deviations. Observed values are reported (23) commercial mixed pellets data.

The average values of 500 simulated plasma concentrations for each time point from 500 simulations were plotted in the mean plots. Vertical bars in the
mean plots represent the standard deviation of 500 simulated concentrations for each time point.

![Graph showing plasma concentration over time for different percentiles](image)

**Figure 2.9** Percentile plot of simulated plasma concentrations of d-amphetamine from mixed immediate-release and enteric-coated pellets in fed subjects. The 25 percent and 75 percent lines represent 25th and 75th percentile of the simulated concentrations, respectively. The 50 percent line represents the median line. The 0 and 100 percent lines show the minimal and maximal simulated concentrations for each time point, respectively. Observed values are reported (23) commercial mixed pellets data.

In the percentile plots, the 25 percent and 75 percent lines represent 25th and 75th percentile values of 500 simulated concentrations at each time point, respectively. This denotes, for each time point, 25% of simulated plasma concentrations are below the 25 percent line and 75% of simulated plasma concentrations are above the 25 percent line. Similarly, 75% of simulated plasma concentrations are below the 75 percent line and 25% of simulated data are above
The 50 percent line represents the median values of 500 simulated concentrations at each time point. The 0 and 100 percent lines show the minimal and maximal simulated concentrations from 500 simulations at each time point, respectively. Therefore, all of simulated concentrations are enclosed between the 0 and 100 percent lines. There is no predicted plasma concentration lie outside the 0 and 100 percent lines. Note that all points on each percentile line are not necessary come from the same simulation trial.

Figure 2.8 shows that the simulated plasma concentration-time curve of amphetamine, after taking into account GI transit time and lag time of emptying in the fed condition, is very close to the reported (23) amphetamine concentrations in plasma following oral administration of commercial mixed pellets in fed subjects. The predicted $C_{\text{max}}$ differs from the observed value by only 2.8 percent (Table 2.3). Most of actual amphetamine concentrations are close to predicted lines, except for the first two points (Figure 2.9). One explanation for the lower concentration of the first two actual data points is that there might be a delay for absorption (lag time) of amphetamine from immediate-release pellets in the fed condition. In the presence of a meal, the drug must dissolve from the pellets and then find its way through the food to absorption surfaces of the stomach or small intestine. Therefore, a short lag time of absorption of the drug from immediate-release pellets in the fed condition is not surprising.

A mean plot and a percentile plot of simulated plasma concentration-time curve of amphetamine from mixed immediate-release and enteric-coated pellets in the fasted condition are presented in Figures 2.10 and 2.11, respectively. Figure 2.10 shows that the simulated plasma concentration-time curve of amphetamine predicts the reported (23) amphetamine concentrations in plasma from commercial mixed pellets in fasted subjects fairly well. The predicted $C_{\text{max}}$ differs from the observed value by 8.4 percent (Table 2.3). The model slightly overestimates the
near-peak concentrations of amphetamine between 4 and 6 hours (Figure 2.11).
While a simulated drug concentration curve within 8.4 percent of specific observed data based on mean literature data is quite good, exact explanations for the difference would require more physiological data in these specific subjects. Somewhat slower gastric emptying in the fasted condition (i.e. longer mean emptying time) and individual variation might play a role in the difference between the observed and the predicted concentrations. Nevertheless, the simulations are quite close to observed data in both the fed and fasted conditions.

Figure 2.10 Mean plot of simulated plasma concentrations of d-amphetamine from mixed immediate-release and enteric-coated pellets in fasted subjects. Vertical bars represent standard deviations. Observed values are reported (23) commercial mixed pellets data.
Figure 2.11  Percentile plot of simulated plasma concentrations of d-amphetamine from mixed immediate-release and enteric-coated pellets in fasted subjects. The 25 percent and 75 percent lines represent 25th and 75th percentile of the simulated concentrations, respectively. The 50 percent line represents the median line. The 0 and 100 percent lines show the minimal and maximal simulated concentrations for each time point, respectively. Observed values are reported (23) commercial mixed pellets data.

Pharmacokinetic models used in the present work incorporated the effect of gastric emptying on plasma concentration-time curve of amphetamine from mixed immediate-release and enteric-coated pellets. The simulations provide numerical and pharmacokinetic support for the intuitive expectation that the plasma concentration-time curve of amphetamine, when administered as mixed immediate-release and enteric-coated pellets both in fed and fasted condition, is not expected to produce a double-pulsed release pattern. Unlike the release of drug in a dissolution chamber, in vivo drug release from enteric-coated pellets is influenced
by both the GI transit and pH in the GI tract. The prolonged input from enteric-coated pellets is expected to be a result of known GI transit characteristics under fed and fasted conditions and that is confirmed by the simulations. For the oral pulsatile delivery system that is a capsule containing an immediate-release tablet and an enteric-coated tablet (16), the GI transit time has no effect on sustaining the drug release from the enteric-coated tablet because a tablet travels in the GI tract as a single unit before disintegrating. Plasma concentration of a drug in such delivery system does provide a double-pulsed pattern unlike pellets that disperse and travel as separate units.

The assumption of the model about instantaneous dissolution of enteric-coated pellets in the intestine seems to be valid, even though the in vitro dissolution profile of the commercial formulation showed that the amphetamine release took about 30 to 45 minutes in pH 6.0 medium. This small “sustaining” release in dissolution is insignificant when compared to the much larger variation of the gastric lag time and emptying. Using assumption of instantaneous dissolution is advantageous in simplifying the model so that it can be applied to other mixed pellet formulations as long as the drug release time is relatively short. One important consideration for using the models to predict other mixed pellet formulations is the type of coating polymer for enteric-coated pellets. Models in present work assume instantaneous dissolution of enteric-coating polymer upon transfer into the intestine. When coating polymer for enteric-coating pellets is the type that does not begin to dissolve in the proximal small intestine or duodenum, the models may not describe pharmacokinetics of the drug well. In such cases, the time required for pellets to move from proximal small intestine to the lower region must be included in the models.
CONCLUSIONS

When a drug is administered as mixed immediate-release and enteric-coated pellets (see model assumptions), plasma concentration-time curve does not mimic that from two immediate-release doses administered at different times. Instead, plasma concentration-time curve from mixed pellets is similar to a typical sustained-release formulation. This evidence supports the idea that prediction of plasma concentrations of drugs from mixed pellet formulation requires pharmacokinetic modeling that takes into consideration gastric emptying and GI transit. Known GI transit characteristics under fed and fasted conditions were applied to previously published pharmacokinetic models and the models were used to predict the plasma concentration-time curve of amphetamine from mixed immediate-release and enteric-coated pellets. The performance of these models was evaluated by Monte Carlo simulations. The assumptions and models are general and can be applied to drugs other than amphetamines. Further work will consider several-compartment models where drug release from enteric-coated pellets is sustained or prolonged but still a first order in the intestine and the resultant impact on drug concentration versus time curves.


CHAPTER 3

PHARMACOKINETIC MODELING AND SIMULATIONS OF GASTROINTESTINAL TRANSIT EFFECTS ON PLASMA CONCENTRATIONS OF DRUGS FROM ENTERIC-COATED PELLET FORMULATIONS—FIRST-ORDER DRUG RELEASE MODELS

Prapoch Watanalumlerd and James W. Ayres
Previous work has shown that plasma concentration-time curves from mixed immediate-release and enteric-coated pellet formulation do not mimic those from two immediate-release doses administered at different times. Instead, plasma concentration-time curves from the mixed pellets are similar to typical sustained-release formulations. The present work focuses on pharmacokinetic models for enteric-coated pellets. One-compartment and two-compartment pharmacokinetic models describing plasma concentrations of drugs from enteric-coated pellets were developed by including gastric emptying time and lag time of emptying, along with other pharmacokinetic parameters of drugs. Developed models were then evaluated by comparing simulated plasma concentrations of erythromycin from Monte Carlo simulations to observed plasma concentrations of erythromycin from available published data of commercial enteric-coated pellet formulation (Eryc®). Results show that effects of gastrointestinal transit on pharmacokinetics of erythromycin administered as enteric-coated pellets can be predicted using the pharmacokinetic models developed herein. The models and assumptions are general and can be applied to other drugs.
INTRODUCTION

When enteric-coated pellet formulations are ingested, the pattern of drug absorption is influenced by many physiological factors such as gastric emptying and food effects (1). Multiple-unit enteric-coated pellets were demonstrated to produce less intrasubject and intersubject variation in absorption than single-unit enteric-coated tablets upon immediate administration after breakfast (2). Gastrointestinal (GI) transit time of drug pellets has been extensively studied. Food was shown to have a profound effect on gastric emptying rate of drug pellets (3-5). Meal size has been shown to have an influence on half-time of gastric emptying of pellets (3, 4). The mean half-time was 78 minutes after a light meal (1500 kJ or 358.5 kcal) compared to 170 minutes for a heavy meal (3600 kJ or 860.4 kcal) (4). In the fasted condition, fifty percent of pellets are emptied from the stomach within an hour with a range of less than 0.3 to 0.9 hour (5) depending upon the time of administration relative to an occurrence of phase 3 of the migrating myoelectric complex (MMC) (6). Patterns of gastric emptying of pellets taken before a meal were shown to be approximately exponential, a typical pattern of gastric emptying of liquid (7).

An initial lag phase has been observed for gastric emptying of solid food with average values ranging from 21 to 60 minutes (5, 8, 9). This lag time reflects primarily the time required to reduce the solid food to smaller sizes (10). After a capsule containing drug pellets was administered in the fed condition, seven of eight subjects showed no gastric emptying of the pellets during the first hour (5). This observed delay of the emptying of pellets suggests that, following capsule disintegration, the pellets became dispersed within the stomach and mixed with food content before being emptied along with the meal (7). During interdigestive
phase (fasted stomach), stomach is less motile and emptying of indigestible solid occurs periodically during phase 3 of MMC (11).

Previous work (Chapter 2) has shown that plasma concentrations of amphetamine from mixed immediate-release and enteric-coated pellet formulation can be described by pharmacokinetic models that take GI transit time into consideration. In this research, pharmacokinetic models are generalized for enteric-coated pellets to accommodate prolonged first-order release rate of drug in the intestine. Both one-compartment and two-compartment models are developed to increase predictive scope. The objective of the present work is to apply pharmacokinetic modeling to predict plasma drug concentrations from prolonged-release enteric-coated pellet formulations using known GI transit information. Monte Carlo simulation is applied to the models to include the effect of GI transit variability on simulated plasma concentrations of drug from prolonged-release enteric-coated pellets. Available pharmacokinetic data of erythromycin enteric-coated pellets in the fed and fasted condition are used to evaluate the models.
MATERIALS AND METHODS

Model Drug

Erythromycin is a weak-basic (pK\textsubscript{a} 8.9) macrolide antibiotic produced by *Streptomyces erythreus*. Erythromycin base, erythromycin stearate, erythromycin ethysuccinate, and erythromycin estolate salts are poorly soluble in water. Erythromycin lactobionate is freely soluble in water. Erythromycin base is highly susceptible to stomach acid inactivation. To protect the drug from acid inactivation, erythromycin base is commercially available in enteric-coating formulations such as enteric-coated tablet (Ery-Tab\textsuperscript{®}), enteric-coated pellets (Eryc\textsuperscript{®}), and enteric-coated particles (PCE\textsuperscript{®} Dispertab) (12). Formulation used in the present study is Eryc\textsuperscript{®} (Warner Chilcott, Inc., NJ). Each capsule contains 250 mg erythromycin base in enteric-coated pellets form.

Pharmacokinetic Models

One-compartment model

Compartmental diagrams illustrating pharmacokinetics of drugs from enteric-coated pellets in the fed and fasted condition are shown in Figures 3.1 and 3.2, respectively. A pre-systemic part of the compartmental diagram in Figure 3.1 is zero-order gastric emptying in the fed condition of enteric-coated pellets into the intestine and first-order absorption of the drug after being released at a first-order rate from the pellets. This rate may be slow to produce prolonged absorption. A
pre-systemic part of the compartmental diagram in Figure 3.2 is first-order gastric emptying in the fasted condition of enteric-coated pellets into the intestine and first order absorption of the drug after being released with first-order rate from the pellets.

**Figure 3.1** Compartmental diagram of one-compartment pharmacokinetic model for enteric-coated pellets in fed condition. $X_{PS}$, amount of drug in pellets form in the stomach; $X_{PI}$, amount of drug in pellets form in the intestine; $X_{SI}$, amount of released drug in the intestine; $X_1$, amount of drug in plasma/blood; $k_0$, a zero-order input of drug into the intestine corresponding to zero-order gastric emptying of enteric-coated pellets in fed condition; $k_r$, a first-order release rate of drug from pellets; $k_a$, a first-order absorption rate constant of drug; $k_{el}$, a first-order elimination rate constant of drug.
Figure 3.2 Compartmental diagram of one-compartment pharmacokinetic model for enteric-coated pellets in fasted condition. \( X_{PS} \), amount of drug in pellets form in the stomach; \( X_{PI} \), amount of drug in pellets form in the intestine; \( X_{SI} \), amount of released drug in the intestine; \( X_{1} \), amount of drug in plasma/blood; \( k_{em} \), a first-order rate of drug input into the intestine corresponding to first-order gastric emptying of enteric-coated pellets in fasted condition; \( k_{r} \), a first-order release rate of drug from pellets; \( k_{a} \), a first-order absorption rate constant of drug; \( k_{el} \), a first-order elimination rate constant of drug.

Differential equations describing compartmental schemes both in fed and fasted conditions are presented in Equations 3.1 to 3.7.

Fed condition

\[
\frac{dX_{PI}}{dt} = k_{0} - k_{r}X_{PI} \quad 3.1
\]

\[
\frac{dX_{SI}}{dt} = k_{r}X_{PI} - k_{a}X_{SI} \quad 3.2
\]

\[
\frac{dX_{1}}{dt} = k_{a}X_{SI} - k_{el}X_{1} \quad 3.3
\]

Fasted condition

\[
\frac{dX_{PS}}{dt} = -k_{em}X_{PS} \quad 3.4
\]
Derivations of mathematical equations describing pharmacokinetics of enteric-coated pellets are extensively presented in Appendices 1 and 2. Derived models for both fed and fasted conditions are summarized below.

Fed condition (One-compartment model)

When $t \leq lag$, $C_1 = 0$.

When $lag < t \leq \tau + lag$,

$$C_1 (lag < t \leq \tau + lag) = \frac{k_0}{V \cdot k_{el}} \left[1 - \frac{k_a k_{el} e^{-k_t (t - lag)}}{(k_a - k_t) (k_{el} - k_t)} - \frac{k_r k_{el} e^{-k_t (t - lag)}}{(k_r - k_a) (k_{el} - k_a) (k_r - k_{el}) (k_a - k_{el})}\right]$$

When $t > \tau + lag$, calculation of $C_1$ is done by summation of Equations 3.9, 3.10, and 3.11.
\[ C_1 (\text{Part 1}) = \frac{k_0 k_a}{V} \left(1 - e^{-k_r t}\right) \left[ \frac{e^{-k_r (t-t_{\text{lag}})}}{(k_a - k_r)(k_{el} - k_r)} + \frac{e^{-k_{el} (t-t_{\text{lag}})}}{(k_r - k_a)(k_{el} - k_a)} \right] \]

\[ C_1 (\text{Part 2}) = \frac{k_a k_0 k_r}{V (k_a - k_{el})} \left[ \frac{1}{k_r k_a} + \frac{e^{-k_r t}}{k_r (k_r - k_a)} - \frac{e^{-k_a t}}{k_a (k_r - k_a)} \right] \left[ e^{-k_{el} (t-t_{\text{lag}})} - e^{-k_a (t-t_{\text{lag}})} \right] \]

\[ C_1 (\text{Part 3}) = \frac{k_0}{V k_{el}} \left[ 1 - \frac{k_a k_{el} e^{-k_r t}}{(k_a - k_r)(k_{el} - k_r)} - \frac{k_r k_{el} e^{-k_a t}}{(k_r - k_a)(k_{el} - k_a)} \right] \frac{k_r k_a e^{-k_{el} t}}{(k_r - k_{el})(k_a - k_{el})} e^{-k_{el} (t-t_{\text{lag}})} \]

Fasted condition (One-compartment model)

When \( t \leq lag \), \( C_1 = 0 \).

When \( t > lag \),

\[ C_1 = \frac{k_r k_{em} k_a D}{V} \left[ e^{-k_{em} (t-t_{\text{lag}})} \right] \left[ \frac{e^{-k_r (t-t_{\text{lag}})}}{(k_a - k_r)(k_r - k_{em})(k_{el} - k_{em})} + \frac{e^{-k_{el} (t-t_{\text{lag}})}}{(k_a - k_r)(k_{em} - k_r)(k_{el} - k_{em}) + \frac{e^{-k_a (t-t_{\text{lag}})}}{(k_r - k_a)(k_{em} - k_a)(k_{el} - k_a)}} \right] \]

where "\( D \)" denotes an enteric-coated dose. "\( V \)" denotes apparent volume of distribution. "\( Lag \)" is lag time of gastric emptying for pellets.
Two-compartment model

Compartmental diagrams illustrating pharmacokinetics of drugs from enteric-coated pellets in fed and fasted condition are presented in Figures 3.3 and 3.4, respectively. A pre-systemic part of the compartmental diagram in Figure 3.3 represents zero-order gastric emptying in the fed condition of enteric-coated pellets into the intestine and first-order absorption of the drug after being released at a first-order rate from the pellets. A pre-systemic part of the compartmental diagram in Figure 3.4 represents first-order gastric emptying in the fasted condition of enteric-coated pellets into the intestine and first order absorption of the drug after being released with first-order rate from the pellets.

Figure 3.3 Compartmental diagram of two-compartment pharmacokinetic model for enteric-coated pellets in fed condition. $X_{PS}$, amount of drug in pellets form in the stomach; $X_{PI}$, amount of drug in pellets form in the intestine; $X_{SI}$, amount of released drug in the intestine; $X_1$, amount of drug in plasma/blood; $k_0$, a zero-order input of drug into the intestine corresponding to zero-order gastric emptying of enteric-coated pellets in fed condition; $k_r$, a first-order release rate of drug from pellets; $k_a$, a first-order absorption rate constant of drug; $k_{12}$ and $k_{21}$, inter-compartmental rate constants; $k_{el}$, a first-order elimination rate constant of drug.
Pellets in the stomach \( \rightarrow \) Pellets in the intestine \( \rightarrow \) Drug solution in the intestine \( \rightarrow \) Blood

**Figure 3.4** Compartmental diagram of two-compartment pharmacokinetic model for enteric-coated pellets in fasted condition. \( X_{PS} \), amount of drug in pellets form in the stomach; \( X_{Pl} \), amount of drug in pellets form in the intestine; \( X_{Sl} \), amount of released drug in the intestine; \( X_1 \), amount of drug in plasma/blood; \( k_{em} \), a first-order rate of drug input into the intestine corresponding to first-order gastric emptying of enteric-coated pellets in fasted condition; \( k_r \), a first-order release rate of drug from pellets; \( k_a \), a first-order absorption rate constant of drug; \( k_{12} \) and \( k_{21} \), inter-compartmental rate constants; \( k_{el} \), a first-order elimination rate constant of drug.

Differential equations describing compartmental schemes both in fed and fasted conditions are presented in Equations 3.13 to 3.21.

**Fed condition**

\[
\frac{dX_{Pl}}{dt} = k_0 - k_r X_{Pl} \tag{3.13}
\]

\[
\frac{dX_{Sl}}{dt} = k_r X_{Pl} - k_a X_{Sl} \tag{3.14}
\]

\[
\frac{dX_1}{dt} = k_a X_{Sl} + k_{21} X_2 - k_{12} X_1 - k_{el} X_1 \tag{3.15}
\]
\[
\frac{dX_2}{dt} = k_{12}X_1 - k_{21}X_2 \quad 3.16
\]

**Fasted Condition**

\[
\frac{dX_{PS}}{dt} = -k_{em}X_{PS} \quad 3.17
\]

\[
\frac{dX_{Pl}}{dt} = k_{em}X_{PS} - k_{r}X_{Pl} \quad 3.18
\]

\[
\frac{dX_{Sl}}{dt} = k_{r}X_{Pl} - k_{a}X_{Sl} \quad 3.19
\]

\[
\frac{dX_{1}}{dt} = k_{a}X_{Sl} + k_{21}X_{2} - k_{12}X_{1} - k_{cd}X_{1} \quad 3.20
\]

\[
\frac{dX_{2}}{dt} = k_{12}X_{1} - k_{21}X_{2} \quad 3.21
\]

Appendices 3 and 4 present derivations of mathematical equations describing two-compartment pharmacokinetic models of enteric-coated pellets in more detail. Derived models for both fed and fasted conditions are summarized below.
Fed condition (Two-compartment models)

When $t \leq \text{lag}$, $C_1 = 0$.

When $\text{lag} < t \leq \tau + \text{lag}$,

$$C_1(\text{lag} \leq t \leq \tau + \text{lag}) = \frac{k_0 k_{21}}{V_1 \alpha \beta} - \frac{k_0 k_a (k_{21} - k_a) e^{-k_f (t - \text{lag})}}{V_1 (k_a - k_f) (\alpha - k_f) (\beta - k_f)}$$

$$- \frac{k_0 k_f (k_{21} - k_f) e^{-k_a (t - \text{lag})}}{V_1 (k_f - k_a) (\alpha - k_a) (\beta - k_a)}$$

$$+ \frac{k_0 k_f k_a (k_{21} - \alpha) e^{-\alpha (t - \text{lag})}}{V_1 \alpha (k_f - \alpha) (k_a - \alpha) (\beta - \alpha)}$$

$$- \frac{k_0 k_f k_a (k_{21} - \beta) e^{-\beta (t - \text{lag})}}{V_1 \beta (k_f - \beta) (k_a - \beta) (\alpha - \beta)}$$

When $t > \tau + \text{lag}$, calculation of $C_1$ is done by summation of Equations 3.23, 3.24, and 3.25.

$$C_1(\text{Part 1}) = \frac{[k_{21} (D_1 + D_2) - D_1 \alpha]}{V_1 (\beta - \alpha)} e^{-\alpha (t - \tau - \text{lag})}$$

$$+ \frac{[k_{21} (D_1 + D_2) - D_2 \beta]}{V_1 (\alpha - \beta)} e^{-\beta (t - \tau - \text{lag})}$$

$$C_1(\text{Part 2}) = \frac{k_a D_3 (k_{21} - \alpha)}{V_1 (k_a - \alpha) (\beta - \alpha)} e^{-\alpha (t - \tau - \text{lag})} + \frac{k_a D_3 (k_{21} - \beta)}{V_1 (k_a - \beta) (\alpha - \beta)} e^{-\beta (t - \tau - \text{lag})}$$

$$+ \frac{k_a D_3 (k_{21} - k_a)}{V_1 (\alpha - k_a) (\beta - k_a)} e^{-k_a (t - \tau - \text{lag})}$$
\[
C_t \text{ (Part 3)} = \frac{k_r k_a D_4 (k_{21} - k_r) e^{-k_r(t - \tau)}}{V_1 (k_a - k_r)(\alpha - k_r)(\beta - k_r)} + \frac{k_r k_a D_4 (k_{21} - k_a) e^{-k_a(t - \tau)}}{V_1 (k_r - k_a)(\alpha - k_a)(\beta - k_a)} + \frac{k_r k_a D_4 (k_{21} - \alpha) e^{-\alpha(t - \tau)}}{V_1 (k_r - \alpha)(\alpha - \alpha)(\beta - \alpha)} + \frac{k_r k_a D_4 (k_{21} - \beta) e^{-\beta(t - \tau)}}{V_1 (k_r - \beta)(\alpha - \beta)}
\]

where "\(V_1\)" denotes apparent volume of distribution. "\(\text{Lag}\)" is lag time of gastric emptying for pellets, and

\[
D_1 = \frac{k_0 k_{21}}{\alpha \beta} \frac{k_0 k_a (k_{21} - k_r) e^{-k_r \tau}}{(k_a - k_r)(\alpha - k_r)(\beta - k_r)} - \frac{k_0 k_r (k_{21} - k_a) e^{-k_a \tau}}{(k_r - k_a)(\alpha - k_a)(\beta - k_a)} - \frac{k_0 k_r k_a (k_{21} - \alpha) e^{-\alpha \tau}}{\alpha (k_r - \alpha)(\alpha - \alpha)(\beta - \alpha)} - \frac{k_0 k_r k_a (k_{21} - \beta) e^{-\beta \tau}}{\beta (k_r - \beta)(\alpha - \beta)}
\]

\[
D_2 = \frac{k_0 k_{12}}{\alpha \beta} \frac{k_0 k_a k_{12} e^{-k_{12} \tau}}{(k_a - k_r)(\alpha - k_r)(\beta - k_r)} - \frac{k_0 k_r k_{12} e^{-k_{12} \tau}}{(k_r - k_a)(\alpha - k_a)(\beta - k_a)} - \frac{k_0 k_r k_a k_{12} e^{-\alpha \tau}}{\alpha (k_r - \alpha)(\alpha - \alpha)(\beta - \alpha)} - \frac{k_0 k_r k_a k_{12} e^{-\beta \tau}}{\beta (k_r - \beta)(\alpha - \beta)}
\]

\[
D_3 = \frac{k_0}{k_a} + \frac{k_0 e^{-k_r \tau}}{(k_r - k_a)} + \frac{k_0 k_r e^{-k_a \tau}}{k_a (k_a - k_r)}
\]

\[
D_4 = \frac{k_0}{k_r} (1 - e^{-k_r \tau})
\]
Fasted condition (Two-compartment models)

When $t \leq \text{lag}$, $C_1 = 0$.

When $t > \text{lag}$,

\[
C_1 = \frac{k_\text{a} k_\text{r} k_\text{em} D}{V_1} \left[ \frac{(k_{21} - k_\text{em}) e^{-k_\text{em}(t-\text{lag})}}{(k_\text{a} - k_\text{em})(k_\text{r} - k_\text{em})(\alpha - k_\text{em})(\beta - k_\text{em})} \right. \\
\left. + \frac{(k_{21} - k_\text{r}) e^{-k_\text{r}(t-\text{lag})}}{(k_\text{a} - k_\text{r})(k_\text{em} - k_\text{r})(\alpha - k_\text{r})(\beta - k_\text{r})} \right. \\
\left. + \frac{(k_{21} - k_\text{a}) e^{-k_\text{a}(t-\text{lag})}}{(k_\text{r} - k_\text{a})(k_\text{em} - k_\text{a})(\alpha - k_\text{a})(\beta - k_\text{a})} \right. \\
\left. + \frac{(k_{21} - \alpha) e^{-\alpha(t-\text{lag})}}{(k_\text{a} - \alpha)(k_\text{r} - \alpha)(k_\text{em} - \alpha)(\beta - \alpha)} \right] + \frac{(k_{21} - \beta) e^{-\beta(t-\text{lag})}}{(k_\text{a} - \beta)(k_\text{r} - \beta)(k_\text{em} - \beta)(\alpha - \beta)}
\]

where "D" denotes an enteric-coated dose.
Model Assumptions

Assumptions for these models are:

1) Pharmacokinetics of the drug are linear in the dosing range of interest. Thus, superposition for determination of plasma drug concentrations can be applied.

2) Enteric-coated formulation is in multi-unit pellet/granule (multi-particulate) form.

3) Upon transfer into the intestine, drug release from enteric-coated pellets in the intestine can be described by a first-order process.

4) Once being released from formulations, the drug is absorbed from the gastrointestinal tract by a first-order process.

5) Pharmacokinetics of the drug are well described by a one-compartment open model or a two-compartment open model.

6) The elimination process is a first-order process.

Monte Carlo Simulations

Pharmacokinetic models above were used in Monte Carlo simulations of plasma concentration-time curves from enteric-coated pellets of erythromycin. One thousand trials for each simulation were performed using MATLAB software, version 6.5 (The MathWorks, Inc., Natick, MA). The simulated plasma concentration-time curves of erythromycin are presented as a mean plot along with its standard deviation and visually compared to published literature data (13-15).
Model Parameters

Post-infusion serum erythromycin concentration declines in a biphasic manner and can be described by a two-compartment pharmacokinetic model (16, 17). The absolute bioavailability (F) of erythromycin from Eryc® capsule was 32 percent (18). Following direct administration of erythromycin solution to the duodenum, the drug was rapidly absorbed with the time to peak concentration of 15 minutes (18). Therefore, intrinsic absorption of erythromycin is not regarded as a rate-limiting step in the absorption process and absorption rate constant value (\( k_a \)) was chosen accordingly. Drug release rate constant (\( k_r \)) was obtained from in vitro dissolution profile of Eryc® (detailed in later section). Since pharmacokinetic parameters of erythromycin from intravenous administration and the absolute bioavailability of oral administration are readily available, the two-compartment model describing pharmacokinetics of drug from enteric-coated pellets appears to be suitable for simulation purposes. Pharmacokinetic parameters of erythromycin base used in the simulations are summarized in Table 3.1.

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>F</th>
<th>V (L)</th>
<th>( \alpha ) (hr(^{-1} ))</th>
<th>( \beta ) (hr(^{-1} ))</th>
<th>( k_{12} ) (hr(^{-1} ))</th>
<th>( k_{31} ) (hr(^{-1} ))</th>
<th>( k_r ) (hr(^{-1} ))</th>
<th>( k_a ) (hr(^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 (fed)</td>
<td>0.32</td>
<td>33.75</td>
<td>1.7</td>
<td>0.50</td>
<td>0.34</td>
<td>1.25</td>
<td>11.6</td>
<td>14.0</td>
</tr>
<tr>
<td>250 (fasted)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Parameters in the models, which represent GI transit effect, are gastric emptying rate constant and lag time of gastric emptying. The gastric emptying rate constant is zero order for the fed condition and first order for the fasted condition.
Variability of Model Parameters

In Monte Carlo simulations, variability of model parameters is included in the simulations. Because effects of gastric emptying on the plasma concentration-time curve are being considered, variability of gastric emptying time and lag time of emptying was included in the simulations. Variability of other model parameters was not included.

Table 3.2  Probability Distribution of Model Parameters and Their Mean and Standard Deviation

<table>
<thead>
<tr>
<th>Parameters (unit)</th>
<th>Distribution</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fed Condition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag time of emptying (hr)</td>
<td>Lognormal</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>Gastric emptying time (hr)</td>
<td>Lognormal</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.97 ± 1.23</td>
</tr>
<tr>
<td><strong>Fasted Condition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag time of emptying (hr)</td>
<td>Lognormal</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>T&lt;sub&gt;50&lt;/sub&gt; (hr)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Lognormal</td>
<td>0.5 ± 0.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Used in simulation of Rutland's data (14).
<sup>b</sup> Used in simulation of Clayton's data (15).
<sup>c</sup> First-order emptying rate constant is calculated from 0.693/T<sub>50</sub>.

Gastric emptying time, lag time of emptying and their variability (standard deviation) were obtained from the literature (3, 4, 8, 11). A lognormal distribution was chosen for all time parameters since time cannot be negative. T<sub>50</sub> is used in calculation of a first-order emptying rate constant in the fasted condition. Lag time
of emptying in the fasted condition was selected based on phase 1, a period of motor inactivity, of MMC, which lasts approximately 30 to 60 min. Variability for lag time of emptying was assumed to be 30 percent. Model parameters and their probability distribution used in the simulations are detailed in Table 3.2.

**Determination of Erythromycin Absorption Profile from Enteric-coated Pellets**

Deconvolution of available erythromycin concentration profiles (13-15) from commercial enteric-coated pellets was performed using WinNonlin Professional software, version 3.2 (Pharsight Corporation, Mountain View, CA), to obtain absorption profiles of erythromycin.

**In Vitro Dissolution Profile of Enteric-coated Pellets**

Commercial enteric-coated erythromycin capsules (Eryc®) containing 250 mg of erythromycin base were used for in vitro dissolution testing. Erythromycin dissolution profile was obtained using USP dissolution apparatus I at 37.5 °C and basket rotating speed at 100 rpm. The formulation without capsule shell was run in simulated gastric fluid (pH 1.4) for 2 hours before being transferred into phosphate buffer medium (pH 6.0). Samples were assayed for erythromycin concentration using an ultraviolet spectrophotometer at 280 nm.
RESULTS AND DISCUSSION

Absorption profiles of erythromycin from deconvolution of plasma concentration profiles of commercial enteric-coated pellets in fed and fasted subjects (13-15) are presented in Figure 3.5. The absorption profiles of erythromycin from enteric-coated pellet formulation look similar to a typical sustained-release formulation with some lag time of absorption. This absorption profile pattern is in agreement with previous findings of mixed immediate-release and enteric-coated pellet formulation as reported in Chapter 2. Lag time of absorption of erythromycin from deconvolution of literature data was about 1 hour and 2 hours in fasted and fed subjects, respectively, and was chosen as lag time of gastric emptying for simulation.

Figure 3.5 Absorption profiles of erythromycin from commercial enteric-coated pellet formulation in fed and fasted subjects.
Simulated plasma concentration-time curves are shown as mean plots. The average values of 1000 simulated plasma concentrations for each time point from 1000 simulations were plotted in the mean plots along with lines showing one standard deviation from mean value.

A mean plot of simulated plasma concentration-time curve of erythromycin from enteric-coated pellets in the fasted condition is presented in Figure 3.6. Simulation results show that the simulated plasma concentration-time curve of erythromycin, after taking into account GI transit time and lag time of emptying in the fasted condition, is very close to the reported (13) erythromycin concentrations following oral administration of commercial enteric-coated pellets. All of average observed concentrations lie within one standard deviation from the mean simulated concentrations.

Figure 3.6 Mean plot of simulated plasma concentrations of erythromycin from enteric-coated pellets in fasted subjects. Observed values (○) are reported (13) commercial enteric-coated pellets (Eryc®) data. Vertical bars represent standard error of mean.
A mean plot of simulated plasma concentration-time curves of erythromycin from enteric-coated pellets in the fed condition are presented in Figures 3.7 and 3.8. Observed data in Figures 3.7 and 3.8 were obtained from different studies. The GI emptying time values used in simulations were different for these data (Table 3.2) to account for different meal type provided in the studies (14, 15). Results from the simulations show that the developed models predict erythromycin concentrations from enteric-coated pellets in fed subjects fairly well. Most of average observed concentrations lie within one standard deviation from the mean simulated concentrations.

![Figure 3.7](image)

**Figure 3.7** Mean plot of simulated plasma concentrations of erythromycin from enteric-coated pellets in fed subjects (●). Observed values (○) are reported (14) commercial enteric-coated pellets (Eryc®) data. Vertical bars represent standard error of mean.
Figure 3.8  Mean plot of simulated plasma concentrations of erythromycin from enteric-coated pellets in fed subjects (II). Observed values (○) are reported (15) commercial enteric-coated pellets (Eryc®) data. Vertical bars represent standard error of mean.

Pharmacokinetic models used in the present work incorporated the effect of GI transit on plasma concentration-time curve of erythromycin from enteric-coated pellets. Consistency of simulation results in the present and previous (Chapter 2) work supports the intuitive expectation that plasma concentration-time curve of drugs, when administered as enteric-coated pellets or as mixed immediate-release and enteric-coated pellets, is similar to that of typical sustained-release formulations. Unlike the release of drug in a dissolution chamber, in vivo drug release from enteric-coated pellets is influenced by GI transit. Prolonged input from enteric-coated pellets is expected to be a result of known GI transit characteristics under fed and fasted conditions.
Even though release rate of erythromycin from Eryc® formulation is first order, it is complete within 15 to 30 minutes after being transferred into dissolution medium pH 6.0. This is relatively rapid dissolution when compared to the much longer time and larger variation of the gastric lag time and emptying. Thus, prolonged drug input into the blood in this case is not due to prolonged release from enteric-coated pellets but is due to transit of pellets from the stomach into the intestine. Drug concentration data from much “slower release” enteric-coated pellets are required for further evaluation of pharmacokinetic models presented in the current work, and currently underway. Another important consideration for using the models is the type of enteric-coating polymer. When coating polymer for enteric-coating pellets is the type that does not begin to dissolve in the proximal small intestine or duodenum, the models may not describe pharmacokinetics of the drug well. In such cases, the time required for pellets to move from proximal small intestine to the lower region must be included in the models.
CONCLUSIONS

Following administration of enteric-coated pellets with relatively rapid first-order release after transit into the intestines, plasma concentration-time curve of drugs is similar to a typical sustained-release formulation. Pharmacokinetic models developed in the present work take into consideration known GI transit characteristics under fed and fasted conditions. The models were used in Monte Carlo simulations to predict plasma concentration-time curve of erythromycin from commercial enteric-coated pellet formulation. Effects of GI transit on pharmacokinetics of erythromycin administered as enteric-coated pellets can be predicted using the developed models. The assumptions of models are general and can be applied to drugs other than erythromycin. Further work is needed for evaluation of the model assumption about first-order drug release from enteric-coated pellets in the intestine using slower release enteric-coated pellets, and is currently underway.
REFERENCES


CHAPTER 4

IN SILICO BIOEQUIVALENCE STUDIES OF MIXED IMMEDIATE RELEASE AND ENTERIC-COATED PELLET FORMULATIONS

Prapoch Watanalumlerd and James W. Ayres
ABSTRACT

The purpose of this research was to estimate probability of mixed immediate-release and enteric-coated pellet formulations in passing bioequivalence (BE) studies using computer simulations. Effect of drug release rate from enteric-coated pellets portion in the intestine for a test formulation and its variability on BE study outcome was studied. Pharmacokinetic models describing plasma concentrations of drug from mixed immediate release and enteric-coated pellet formulations were used in Monte Carlo simulations of BE studies. Amphetamine was chosen as a model drug. A design of simulated BE studies was a two-treatment crossover design for single-dose studies. Plasma concentration profiles were generated from parameters with random variability. Intersubject and intrasubject variability of pharmacokinetic parameters were set at 25% and 15%, respectively. Intersubject and intrasubject variability of GI transit parameters were set at 30% and 20%, respectively. Residual variability was set at 5%. Statistical analysis based on the two one-sided tests procedure for peak drug concentration ($C_{\text{max}}$) parameter was used to determine bioequivalency of the reference and test formulations. Two thousand (2000) BE studies were simulated for each combination of parameters' values and variability in both fed and fasted conditions. Probability of passing BE study was calculated for each parameter combination. Simulation results show that probability of passing BE studies is similar for both fasted and fed conditions. The probability of passing BE studies depends on both drug release rate ($k_r$) of enteric-coated pellets and number of subjects for the studies. The probability of passing BE studies was also found to be insensitive to moderate variability of $k_r$ if appropriate $k_r$ value for the test formulation was used.
INTRODUCTION

For orally administered drug products, bioequivalence (BE) studies generally aim to demonstrate differences or similarities between products using measurement of peak (peak drug concentration, \( C_{\text{max}} \)) and total exposure (area under the concentration-time curve, \( \text{AUC} \)) (1). BE studies are a critical part of abbreviated new drug application (ANDA) submissions. The purpose is to demonstrate presence or absence of BE between pharmaceutically equivalent products (i.e. generic products and innovators' products) (1). BE studies are also used during some periods of investigational new drug applications (INDs) and new drug applications (NDAs) as well as during post-approval changes (1).

To determine whether the test and reference products are comparable, the US Food and Drug Administration (FDA) recommended that the average bioequivalence statistical analysis for pharmacokinetic measures mentioned above (\( C_{\text{max}} \) and \( \text{AUC} \)) be based on the two one-sided tests procedure (2). The two one-sided tests procedure was described in the work of Schuirmann, D.J. (3).

For a two-period design, a minimum number of 12 healthy volunteers will generally be included in a BE study. To allow for dropouts and to increase power of statistical analysis, more volunteers are commonly recruited. Because considerable amounts of resources are invested in carrying out BE studies, selection of final test formulation or product needs to be carefully thought out.

In silico (computer-based simulation) BE studies have been widely used as a tool to evaluate likelihood of passing BE studies for drug products and to investigate effects of several variables on BE study outcomes (4-9). Chapters 2 and 3 of the current thesis research discussed how gastrointestinal (GI) transit affects pharmacokinetics of drugs from various enteric-coated pellet formulations.
Pharmacokinetic models describing plasma concentration-time curve of drugs from enteric-coated pellet formulations have been proposed. Due to the influence of GI transit on absorption kinetics of drug from enteric-coated pellets, it is now proposed that dissolution profile of the test formulation need not match that of the reference formulation in order to be bioequivalent. Computer simulation is used to substantiate this concept. The present research focuses on mixed immediate release and enteric-coated pellet formulations. Commercial mixed immediate release and enteric-coated pellet formulation of mixed amphetamine salts (Adderall XR™) was used as a reference formulation. The objective is to study effect of drug release rate from enteric-coated pellets in the intestine for a test formulation and its variability on probability of passing in silico BE studies. Pharmacokinetic models describing plasma concentrations of drug from mixed immediate release and enteric-coated pellet formulations were used in Monte Carlo simulations.
MATERIALS AND METHODS

Pharmacokinetic Models

Commercial mixed immediate release and enteric-coated pellet formulation of amphetamine (Adderall XR™) was used as a reference formulation. Adderall XR™ comprises equal amounts of mixed amphetamine salts in immediate-release pellets form and enteric-coated pellets form. These mixed amphetamine salts consist of d-amphetamine sulfate, d-amphetamine saccharate, dl-amphetamine sulfate, and dl-amphetamine aspartate. Mixed immediate release and enteric-coated pellets of various amphetamine release rates were used as test formulations. Pharmacokinetic models for mixed immediate release and enteric-coated pellets of amphetamine are used throughout the present research. The models are similar to those described in Chapter 2 of the current thesis research. One modification is that enteric-coated pellets are allowed to have slower release rate in the intestine. Therefore, a first-order release rate of drug from pellets (k_r) is added to the enteric-coated pellets model making this part identical to the one described in Chapter 3. Compartmental diagrams of the models for fed and fasted conditions are shown in Figures 4.1 and 4.2, respectively. Mathematical equations describing plasma/blood concentrations of amphetamine from mixed immediate release and enteric-coated pellets used for simulations of BE studies are presented in Equations 4.1 to 4.8.
Immediate Release Pellets

\[ D_{IR} \rightarrow X_1 \rightarrow k_{el} \]

Drug in the GI tract

Blood

Enteric-Coated Pellets

\[ D_{EC} \rightarrow X_{PI} \rightarrow X_{SI} \rightarrow X_1 \rightarrow k_{el} \]

Pellets in the stomach

Pellets in the intestine

Drug solution in the intestine

Blood

**Figure 4.1** Compartmental diagrams of pharmacokinetic models for in silico bioequivalence studies in fed condition.

Immediate Release Pellets

\[ D_{IR} \rightarrow X_1 \rightarrow k_{el} \]

Drug in the GI tract

Blood

Enteric-Coated Pellets

\[ D_{EC} \rightarrow X_{PI} \rightarrow X_{SI} \rightarrow X_1 \rightarrow k_{el} \]

Pellets in the stomach

Pellets in the intestine

Drug solution in the intestine

Blood

**Figure 4.2** Compartmental diagrams of pharmacokinetic models for in silico bioequivalence studies in fasted condition.
Symbol notation: $X_{PI}$, amount of drug in pellets form in the intestine; $X_{SI}$, amount of released drug in the intestine; $X_1$, amount of drug in plasma/blood; $D_{IR}$, an immediate release dose; $D_{EC}$, an enteric-coated dose; $k_0$, a zero-order input of drug corresponding to zero-order gastric emptying of enteric-coated pellets in fed condition; $k_{em}$, a first-order rate of drug input into the intestine corresponding to first-order gastric emptying of enteric-coated pellets in fasted condition; $k_r$, a first-order release rate of drug from pellets; $k_a$, a first-order absorption rate constant of drug; $k_{el}$, a first-order elimination rate constant of drug.

Models for fed condition

When $t \leq \text{lag}$,

$$C_1 = \frac{k_a D_{IR}}{V(k_a - k_{el})} \left( e^{-k_{el}t} - e^{-k_a t} \right)$$

When $\text{lag} < t \leq T + \text{lag}$,

$$C_1(\text{lag} < t \leq \tau + \text{lag}) = \frac{k_a D_{IR}}{V(k_a - k_{el})} \left( e^{-k_{el}t} - e^{-k_a t} \right)$$

$$+ \frac{k_0}{V \cdot k_{el}} \left[ 1 - \frac{k_a k_{el} e^{-k_r(t-\text{lag})}}{(k_a - k_r)(k_{el} - k_r)} \right]$$

$$- \frac{k_r k_{el} e^{-k_a(t-\text{lag})}}{(k_r - k_a)(k_{el} - k_a)} - \frac{k_r k_a e^{-k_{el}(t-\text{lag})}}{(k_r - k_{el})(k_a - k_{el})}$$
When \( t > \tau + \text{lag} \), calculation of \( C_1 \) is done by summation of Equations 4.3, 4.4, 4.5, and 4.6.

\[
C_1 (\text{Part 1}) = \frac{k_a D_{IR}}{V(k_a - k_{cl})} \left( e^{-k_{el}t} - e^{-k_{a}t} \right) \tag{4.3}
\]

\[
C_1 (\text{Part 2}) = \frac{k_0 k_a}{V} \left( 1 - e^{-k_{i}t} \right) \left[ \frac{e^{-k_i(t-\tau-\text{lag})}}{(k_a - k_r)(k_{el} - k_r)} \right. \\
+ \frac{e^{-k_{el}(t-\tau-\text{lag})}}{(k_r - k_a)(k_{el} - k_a)} \\
\left. + \frac{e^{-k_{el}(t-\tau-\text{lag})}}{(k_r - k_{el})(k_a - k_{el})} \right] \tag{4.4}
\]

\[
C_1 (\text{Part 3}) = \frac{k_a k_0 k_r}{V(k_a - k_{el})} \left[ \frac{1}{k_r k_a} + \frac{e^{-k_{r}t}}{k_{r} (k_r - k_a)} \right. \\
- \frac{e^{-k_{a}t}}{k_a (k_r - k_a)} \left[ e^{-k_{el}(t-\tau-\text{lag})} - e^{-k_{a}(t-\tau-\text{lag})} \right] \tag{4.5}
\]

\[
C_1 (\text{Part 4}) = \frac{k_0}{V k_{el}} \left[ 1 - \frac{k_a k_{el}e^{-k_{r}t}}{(k_a - k_r)(k_{el} - k_r)} - \frac{k_r k_{el}e^{-k_{a}t}}{(k_r - k_a)(k_{el} - k_a)} \right. \\
- \frac{k_r k_{el}e^{-k_{el}t}}{(k_r - k_{el})(k_a - k_{el})} \left. \right] e^{-k_{el}(t-\tau-\text{lag})} \tag{4.6}
\]

Models for fasted condition

When \( t \leq \text{lag} \),

\[
C_1 = \frac{k_a D_{IR}}{V(k_a - k_{cl})} \left( e^{-k_{el}t} - e^{-k_{a}t} \right) \tag{4.7}
\]
When $t > \text{lag}$,

$$C_t = \frac{k_a D_{IR}}{V(k_a - k_{el})} \left( e^{-k_{el}t} - e^{-k_r t} \right)$$

$$+ \frac{k_r k_{em} D_{EC}}{V} \left( \frac{e^{-k_{em}(t-\text{lag})}}{(k_a - k_{em})(k_r - k_{em})(k_{el} - k_{em})} \right)$$

$$+ \frac{(k_a - k_r)(k_{em} - k_r)(k_{el} - k_r)}{e^{-k_r(t-\text{lag})} \cdot (k_a - k_{el})(k_r - k_{el})(k_{em} - k_{el})}$$

where "\(V\)" denotes apparent volume of distribution. "\(\text{Lag}\)" is lag time of gastric emptying for enteric-coated pellets.

**Model Assumptions**

Assumptions for these models are:

1) Pharmacokinetic of the drug is linear in the dosing range of interest. Thus, superposition for determination of plasma drug concentrations can be applied.

2) Enteric-coated portion of formulations is in multiple-unit pellet/granule (multi-particulate) form.

3) Upon transfer into the intestine, the drug is released from enteric-coated pellets with a first-order rate $k_r$.

4) Once being released from formulations, the drug is absorbed from the gastrointestinal tract by a first-order process.

5) Pharmacokinetic of the drug after absorption is described by a one-compartment open model.
6) The elimination process is a first-order process.

**Statistical Models**

All pharmacokinetic and GI transit parameters are assumed to be log-normally distributed around the population means as in Equation 4.9.

\[
\phi_{ij} = \phi \cdot e^{\eta_{ij}} \cdot e^{\varepsilon_{ij}}
\]  

where \( \phi_{ij} \) is the parameter of the \( j^{th} \) individual from the \( i^{th} \) formulation, \( \phi \) is the population parameter, \( \eta_{ij} \) represents intersubject variability, and \( \varepsilon_{ij} \) represents intrasubject variability.

Residual error (e.g. assay error, model misspecification) is assumed to be proportional to plasma concentrations. Thus, observed plasma concentrations can be expressed as:

\[
\left[ C_{ij}(t) \right]_{\text{obs}} = \left[ C_{ij}(t) \right] \cdot (1 + \varepsilon_{\text{res}})
\]  

where \( \varepsilon_{\text{res}} \) represents the residual error.

**Monte Carlo Simulations**

Simulations of BE data and BE analyses of mixed immediate release and enteric-coated pellets formulation of amphetamine were performed using MATLAB software, version 6.5 (The MathWorks, Inc., Natick, MA). Examples of
M-script (MATLAB programming codes) for the simulations of BE studies are given in Appendix 5.

Data simulation

A design of simulated BE studies was a two-treatment crossover design for single-dose (20 mg) studies. Sampling times for each simulated study were 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, and 24 hours. Plasma concentration profiles were generated from parameters with random variability as mentioned in the “Pharmacokinetic Models” and “Statistical Models” sections. Intersubject and intrasubject variability of pharmacokinetic parameters were set at 25% and 15%, respectively. Intersubject and intrasubject variability of GI transit parameters were set at 30% and 20%, respectively. Residual variability was set at 5%. Two scenarios based on objectives of the study were explored.
Scenario I

Effects of number of subjects (N) and drug release rate in the intestine (kr) for the test formulation on the probability of passing or failing BE studies were investigated.

Scenario II

Effects of variability of kr for the test formulation on the probability of passing or failing BE studies were investigated.

A summary of parameters and different levels of variability used in simulations is given in Table 4.1.

Drug release rate constant (kr) values of 0.36, 0.51, 0.69, 0.92, 1.2, 1.6, and 3.5 hr⁻¹ in Table 4.1 represent drug release from enteric-coated pellets equivalent to 30, 40, 50, 60, 70, 80, and 97 percent, respectively, at 1 hour in a buffer dissolution medium (pH 6.0).

For each combination of parameters' value and variability in both fed and fasted conditions, 2000 BE studies were simulated. Probability of passing BE study was calculated for each parameter combination. There were 36 parameter combinations in scenario I and 32 parameter combinations in scenario II. Thus, total of 136,000 BE studies were simulated.
Table 4.1  Summary of Parameter Values and Their Level of Variability for In Silico Bioequivalence Studies

<table>
<thead>
<tr>
<th>Parameters (Unit)</th>
<th>Mean Values</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pharmacokinetic parameters of amphetamine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose (mg)</td>
<td>20 (D_{IR} 10 mg, D_{EC} 10 mg)</td>
<td>-</td>
</tr>
<tr>
<td>$k_a$ (hr$^{-1}$)</td>
<td>0.744</td>
<td>25% (intersubject)</td>
</tr>
<tr>
<td>$k_d$ (hr$^{-1}$)</td>
<td>0.067</td>
<td>15% (intrasubject)</td>
</tr>
<tr>
<td>V/F (L)</td>
<td>247</td>
<td></td>
</tr>
<tr>
<td><strong>GI transit parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag time of emptying (hr)</td>
<td>1 (fed)</td>
<td>30% (intersubject)</td>
</tr>
<tr>
<td></td>
<td>0.75 (fasted)</td>
<td>20% (intrasubject)</td>
</tr>
<tr>
<td>Gastric emptying time in fed (hr)</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>$k_{em}$ in fasted (hr$^{-1}$)</td>
<td>1.39</td>
<td></td>
</tr>
<tr>
<td>Residual variability (error)</td>
<td></td>
<td>5%</td>
</tr>
<tr>
<td><strong>Scenario I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>12, 19, 24</td>
<td>-</td>
</tr>
<tr>
<td>$k_r$ (hr$^{-1}$)</td>
<td>3.5 (reference); 0.36, 0.51, 0.69, 0.92, 1.2, 1.6 (test)</td>
<td>5%</td>
</tr>
<tr>
<td><strong>Scenario II</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>$k_r$ (hr$^{-1}$)</td>
<td>3.5 (reference)</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>0.51, 0.69 (test)</td>
<td>5, 10, 20, 30, 40, 50, 70, 90%</td>
</tr>
</tbody>
</table>
Bioequivalence Analysis

To conclude bioequivalency of the reference and test formulations, the statistical analysis based on the two one-sided tests procedure (3) for \( C_{\text{max}} \) parameter was performed. Briefly, plasma concentration profiles from simulations were used to determine \( C_{\text{max}} \). The \( C_{\text{max}} \) data from each subject were log-transformed and analyzed under the normality assumption. Two sets of one-sided hypotheses (3) are given as:

Null hypothesis I

\[ H_{01}: \mu_T - \mu_R \leq \theta_1 \]

Alternative hypothesis I

\[ H_{a1}: \mu_T - \mu_R > \theta_1 \]

Null hypothesis II

\[ H_{02}: \mu_T - \mu_R \geq \theta_2 \]

Alternative hypothesis II

\[ H_{a2}: \mu_T - \mu_R < \theta_2 \]

where \( \theta_1 \) (0.8) and \( \theta_2 \) (1.25) are BE limits. \( \mu_T \) and \( \mu_R \) are average responses of log-transformed measure for the test and reference formulations, respectively.

The two-sided tests procedure is identical to a procedure of declaring bioequivalency if 90% confidence interval for \( \mu_T - \mu_R \) is contained in BE limits \([\theta_1, \theta_2]\) (3). The 90% confidence interval was calculated using analysis of variance (ANOVA) for a two-treatment crossover design.
RESULTS AND DISCUSSION

Simulations of BE studies for mixed enteric-coated pellets formulations containing 10 mg amphetamine salts in immediate-release pellet portion and 10 mg amphetamine salts in enteric-coated pellet portion have been performed. Figures 4.3 to 4.8 illustrate plasma concentration-time profiles from one simulated bioequivalence study in 12 fasted subjects. Figures 4.9 to 4.14 illustrate plasma concentration-time profiles from one simulated bioequivalence study in 12 fed subjects.

The influence of number of subjects (N) and drug release rate in the intestine (kr) for the test formulation on the probability of passing BE studies, using amphetamine as a model drug, is illustrated in Figure 4.15. The probability of passing BE studies for test formulations depends on both kr and N. For most cases, the probability of passing BE studies stays fairly constant when percent drug release in the intestine is faster than 50 to 60 percent within the first hour. The probability of passing then drops very rapidly when drug release in the intestine is slower than 50 percent for the first hour. When 12 subjects were used in simulations, the probability of passing barely approaches 95 percent regardless of kr, thus, suggesting that more subjects should be included to ensure high probability of passing. The probability of passing BE studies appears to be similar for both fasted and fed conditions.
Figure 4.3 Example of plasma concentration-time profiles from one simulated bioequivalence study in 12 fasted subjects ($k_{r_{(t_{esr})}} = 0.36 \, \text{hr}^{-1}$, $N = 12$). a) Individual plasma profiles from Test formulation. b) Individual plasma profiles from Reference formulation. c) Mean plasma profiles.
Figure 4.4 Example of plasma concentration-time profiles from one simulated bioequivalence study in 12 fasted subjects (k_{\text{r,test}} = 0.51 \text{ hr}^{-1}, N = 12). a) Individual plasma profiles from Test formulation. b) Individual plasma profiles from Reference formulation. c) Mean plasma profiles.
Figure 4.5  Example of plasma concentration-time profiles from one simulated bioequivalence study in 12 fasted subjects ($k_{r, test} = 0.69$ hr$^{-1}$, $N = 12$). a) Individual plasma profiles from Test formulation. b) Individual plasma profiles from Reference formulation. c) Mean plasma profiles.
Figure 4.6  Example of plasma concentration-time profiles from one simulated bioequivalence study in 12 fasted subjects ($k_r (\text{test}) = 0.92 \text{ hr}^{-1}, N = 12$). a) Individual plasma profiles from Test formulation. b) Individual plasma profiles from Reference formulation. c) Mean plasma profiles.
Figure 4.7 Example of plasma concentration-time profiles from one simulated bioequivalence study in 12 fasted subjects ($k_{r\text{(test)}} = 1.2 \text{ hr}^{-1}, N = 12$). a) Individual plasma profiles from Test formulation. b) Individual plasma profiles from Reference formulation. c) Mean plasma profiles.
Figure 4.8  Example of plasma concentration-time profiles from one simulated bioequivalence study in 12 fasted subjects (k_{r(test)} = 1.6 hr^{-1}, N = 12). a) Individual plasma profiles from Test formulation. b) Individual plasma profiles from Reference formulation. c) Mean plasma profiles.
Figure 4.9 Example of plasma concentration-time profiles from one simulated bioequivalence study in 12 fed subjects ($k_{r\text{test}} = 0.36 \text{ hr}^{-1}, N = 12$). a) Individual plasma profiles from Test formulation. b) Individual plasma profiles from Reference formulation. c) Mean plasma profiles.
Figure 4.10 Example of plasma concentration-time profiles from one simulated bioequivalence study in 12 fed subjects ($k_r_{(test)} = 0.51 \text{ hr}^{-1}, N = 12$). a) Individual plasma profiles from Test formulation. b) Individual plasma profiles from Reference formulation. c) Mean plasma profiles.
Figure 4.11 Example of plasma concentration-time profiles from one simulated bioequivalence study in 12 fed subjects ($k_{r (test)} = 0.69 \text{ hr}^{-1}$, $N = 12$). a) Individual plasma profiles from Test formulation. b) Individual plasma profiles from Reference formulation. c) Mean plasma profiles.
Figure 4.12 Example of plasma concentration-time profiles from one simulated bioequivalence study in 12 fed subjects ($k_f$ (test) = 0.92 hr$^{-1}$, N = 12). a) Individual plasma profiles from Test formulation. b) Individual plasma profiles from Reference formulation. c) Mean plasma profiles.
Figure 4.13  Example of plasma concentration-time profiles from one simulated bioequivalence study in 12 fed subjects ($k_{r \text{ (test)}} = 1.2 \text{ hr}^{-1}, N = 12$). a) Individual plasma profiles from Test formulation. b) Individual plasma profiles from Reference formulation. c) Mean plasma profiles.
Figure 4.14 Example of plasma concentration-time profiles from one simulated bioequivalence study in 12 fed subjects ($k_t$ (test) = 1.6 hr$^{-1}$, N = 12). a) Individual plasma profiles from Test formulation. b) Individual plasma profiles from Reference formulation. c) Mean plasma profiles.
Figure 4.15 Influence of N and $k_r$ for the test formulation on the probability of passing bioequivalence studies in fasted and fed conditions.

Simulation results for effects of N and $k_r$ on the probability of passing BE studies are tabulated in Table 4.2.
Table 4.2  Summary of Effects of N and k₁ for the Test Formulation on the Probability of Passing Bioequivalence Studies

<table>
<thead>
<tr>
<th>N</th>
<th>%Drug release at 1 hr in buffer pH 6.0</th>
<th>Probability of passing BE studies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fasted</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td></td>
<td>0.999</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td>0.998</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>0.995</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>0.982</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>0.911</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>0.594</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td></td>
<td>0.994</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td>0.994</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>0.982</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>0.958</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>0.863</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>0.536</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td></td>
<td>0.951</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td>0.946</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>0.920</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>0.856</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>0.710</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>0.402</td>
</tr>
</tbody>
</table>

To achieve 95 percent or higher probability of passing BE studies, 19 or more subjects, after accounting for dropouts, should be included. In addition, percent drug release of the test formulation should be at least 50 percent within the first hour in buffer (pH 6.0).

The influence of variability of k₁ for the test formulation on the probability of passing BE studies is illustrated in Figure 4.16. Twenty-four subjects were used.
in these simulations. Two different percent drug release rates (in buffer within the first hour) for test formulations were chosen (50 percent versus 40 percent). Simulation results show that the probability of passing decreases as variability of $k_r$ becomes larger. When percent drug release for test formulations is set at 50 percent, the probability of passing BE studies stays well above 95 percent if coefficient of variation (%CV) of $k_r$ is smaller than 50 percent. This finding suggests that BE study result is not sensitive to moderate variability of $k_r$ if appropriate $k_r$ value for the test formulation is used. Summary of effects of variability of $k_r$ for the test formulation on the probability of passing BE studies is presented in Table 4.3.

![Graph showing the probability of passing vs. %CV of $k_r$](image)

**Figure 4.16** Influence of variability of $k_r$ for the test formulation on the probability of passing BE studies in fasted and fed conditions (N = 24). %CV, coefficient of variation.
Table 4.3  Summary of Effects of Variability of $k_r$ for the Test Formulation on the Probability of Passing Bioequivalence Studies

<table>
<thead>
<tr>
<th>%Drug release at 1 hr in buffer pH 6.0</th>
<th>%CV of $k_r$ for test formulations</th>
<th>Probability of passing BE studies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fasted</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fed</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.970</td>
<td>0.976</td>
</tr>
<tr>
<td>10</td>
<td>0.980</td>
<td>0.978</td>
</tr>
<tr>
<td>20</td>
<td>0.972</td>
<td>0.976</td>
</tr>
<tr>
<td>30</td>
<td>0.974</td>
<td>0.966</td>
</tr>
<tr>
<td>40</td>
<td>0.974</td>
<td>0.964</td>
</tr>
<tr>
<td>50</td>
<td>0.954</td>
<td>0.953</td>
</tr>
<tr>
<td>70</td>
<td>0.924</td>
<td>0.926</td>
</tr>
<tr>
<td>90</td>
<td>0.872</td>
<td>0.863</td>
</tr>
<tr>
<td>40</td>
<td>0.906</td>
<td>0.905</td>
</tr>
<tr>
<td>5</td>
<td>0.911</td>
<td>0.908</td>
</tr>
<tr>
<td>10</td>
<td>0.899</td>
<td>0.894</td>
</tr>
<tr>
<td>20</td>
<td>0.886</td>
<td>0.886</td>
</tr>
<tr>
<td>30</td>
<td>0.869</td>
<td>0.866</td>
</tr>
<tr>
<td>40</td>
<td>0.838</td>
<td>0.856</td>
</tr>
<tr>
<td>50</td>
<td>0.746</td>
<td>0.776</td>
</tr>
<tr>
<td>70</td>
<td>0.664</td>
<td>0.670</td>
</tr>
<tr>
<td>90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = 24  
%CV, coefficient of variation

In the present research, only $C_{\text{max}}$, not AUC, was used for making decision about bioequivalency. The justification for using only $C_{\text{max}}$ lies upon the assumptions of amphetamine pharmacokinetic models used in simulations. Linear pharmacokinetics was assumed for the models regardless of meal condition and drug release rate of formulations. Therefore, $C_{\text{max}}$ is the parameter that will be
affected by varying rate of absorption due to changing in meal condition and drug release rate of the formulations. As a result, $C_{\text{max}}$ is a more sensitive measure than AUC for concluding bioequivalency of formulation of interest, mixed immediate release and enteric-coated pellets. Determination of whether or not any changes made to the formulations will affect bioequivalency can then be done by examining the effect on $C_{\text{max}}$.

It should be noted that the statistical analysis for BE study used in the present research does not include testing for period or sequence effects. The analysis assumed adequate washout time between treatment periods of BE studies. Since there is no existing evidence of these effects in amphetamine studies, such statistical analysis for BE studies is considered sufficient for the purpose of this research.

The present research demonstrates another application of pharmacokinetic models for enteric-coated pellets previously presented in Chapters 2 and 3. Results of in silico BE studies from the present research are very useful when designing generic products of mixed pellet formulations or modifying existing formulations. It implies that new formulations need not exactly match dissolution profile of the existing formulations so long as enteric-coated pellets for both formulations start releasing the drug in the same region of the intestine. Simulation of BE studies presented here is also useful for refining or selecting the final product for actual BE study.
Pharmacokinetic models describing plasma concentrations of drug from mixed immediate release and enteric-coated pellet formulations were used to perform Monte Carlo simulations of BE studies. Effect of amphetamine release rate ($k_r$) from enteric-coated pellets in the intestine for test formulations and variability of release rate on probability of passing in silico BE studies has been studied. Probability of passing BE studies for test formulations depends on both $k_r$ and number of subjects. The probability of passing BE studies remains fairly constant when percent drug release in the intestine is faster than 50 to 60 percent within the first hour, but drops very rapidly as the release rate becomes slower. The probability of passing BE studies is similar for both fasted and fed conditions. The probability of passing BE studies was found to be insensitive to moderate variability of $k_r$ if appropriate $k_r$ value (i.e. percent drug release at 1 hour in the intestine for test formulations of 50 percent or faster) for the test formulation was used. Results from the present research are very useful in designing generic products of mixed pellet formulation and in refining or selecting the final product for actual BE study.
REFERENCES


CHAPTER 5

DEVELOPMENT OF CRUSHABLE ENTERIC-COATED FORMULATIONS

Prapoch Watanalumlerd and James W. Ayres
ABSTRACT

Crushable enteric-coated formulations of amphetamine multi-layered spray-coated enteric-coated drug pellets were prepared, and then compressed into crushable tablets. All studied crushable enteric-coated pellets consisted of an innermost amphetamine layer, enteric-coating layer(s), and hydrophilic polymer layer(s). Hydrophilic polymer layer provide abilities to self-seal cracks caused to the coating film by compacting and crushing of the pellets, thus preventing the formulations from losing controlled-release properties.

In vitro drug release profiles of studied formulations were obtained using the USP XXV dissolution apparatus II, paddle stirring method, with a paddle rotation speed of 100 rpm and dissolution bath temperature of 37.5°C.

Polyox® N-80 at studied amount (5%) is below the borderline amount required for protecting the tested formulations against compaction and crushing sufficiently to prevent any drug release in two hours of dissolution testing in gastric fluid. Improved sealant effect may be accomplished by increasing the amount of Polyox® in the formulation. Eudragit® L 30 D-55 with pectin and Aquacoat® ECD-30 with pectin provide some protection against small compaction forces causing by direct compression of dry-blend of pellets and disintegrant mix. Combination of Polyox® and Aquacoat® with pectin was shown to have good protective properties against compaction and crushing.

Even though none of the studied formulations were able to fulfill USP XXV requirements regarding maximum amount of drug release allowed in acidic medium at 2 hours, which is limited to 10% or less, results from the present research are very promising for future development of crushable/chewable enteric-coated formulations.
INTRODUCTION

Multi-unit pellet dosage forms present several advantages over single-unit dosage forms such as tablets. They can be distributed throughout the gastrointestinal tract; therefore, improving drug absorption and producing less risk of local irritation (1). Gastrointestinal (GI) transit and influence of food tend to be less variable for multi-unit dosage forms (2, 3); thus, better predictability in therapeutic effect can be expected.

The need as well as the difficulties of compressing drug-containing pellets into single-unit dosage forms such as tablets, wherein the pellets have controlled-release properties, has been well reviewed (4). Examples of problems associated with making single-unit dosage forms from controlled-release pellets are loss of sustained-release properties after compaction, excessive use of tableting excipient, and segregation of drug pellets from dry powder of tableting excipient. Research involving multiple-coated self-sealing sustained-release formulations as solutions to these problems have been reported (4-6).

The present research recognized a need to formulate fast disintegrating single-unit enteric-coated multi-units from compaction of enteric-coated pellets. The product, made from the multiple-layer enteric-coated pellets, was expected to have a more reliable and predictable absorption than a traditional enteric-coated tablet (7). The process of making a single-unit dosage form (tablet) from compaction of multiple-unit enteric-coated pellets to produce similar in vivo behavior to the same enteric-coated pellets in a non-compacted form in a capsule requires the tablet to quickly disintegrate into multiple-unit pellets as soon as ingested. The process also involves protecting individual pellets from losing enteric-coating properties after compaction. That is, the drug must not be released
from the pellets into gastric fluid. This goal may be accomplished by the use of hydrophilic polymer(s) (e.g. polyethylene oxide) that will function as sealant for cracks in enteric-coating layer(s) following compaction as detailed in U.S. Patent 5,766,623 (4).

Enteric polymer chosen for this research was methacrylic acid copolymer (Eudragit® L 30 D-55). Eudragit® L 30 D-55 is a pH dependent anionic aqueous polymer dispersion that solubilizes above pH 5.5 (approximate duodenal pH). Therefore, enteric-coated pellets made using this polymer will generally release drug as soon as they arrive at the duodenum. Beckert et al. has shown that there were difficulties of making disintegrating tablets from compression of enteric-coated pellets produced using Eudragit® L 30 D-55, even at high amounts of enteric coat (up to 25% w/w) (8). According to the United States Pharmacopeia (USP) XXV, the amount of released drug from typical enteric-coated formulations allowed in acidic medium after 2 hours of dissolution is limited to 10% or less (9). Thus, this limit was taken as the minimum goal to be achieved in the current research.

The objective of this research is to produce self-sealing enteric-coated crushable tablet formulations that maintain enteric-coating properties regardless of compaction or crushing.
MATERIALS AND METHODS

Chemicals

All chemicals used in this study were obtained from standard sources. Amphetamine salts (dl-Amphetamine sulfate, dl-Amphetamine aspartate, d-Amphetamine sulfate, d-Amphetamine saccharate) were received from Teva Pharmaceuticals, PA. Dibutyl sebacate was purchased from Sigma Chemicals Co., St. Louis, MO. Ethylcellulose aqueous dispersion (Aquacoat® ECD-30) from FMC Corporation, Philadelphia, PA. Hydroxypropyl cellulose (Klucel®) EXF from Aqualon, Wilmington, DE. Hydroxypropyl methylcellulose (HPMC) E5 was received from Teva Pharmaceuticals, PA. Lactose monohydrate was purchased from J.T. Baker Chemical Co., Phillipsburg, NJ. Methacrylic acid copolymer (Eudragit® L 30 D-55) was obtained from Röhm Tech Inc., Malden, MA. Pectin from Sigma Chemical Co., St. Louis, MO. Polyethylene oxide (Polyox®) N-80 (MW. 200,000) from Union Carbide Corporation, Danbury, CT. Polyvinylpyrrolidone (PVP) K-30 (MW. 40,000) from Spectrum Quantity Products, Inc., New Brunswick, NJ. Sodium chloride, Sodium phosphate tribasic, Concentrated hydrochloric acid were purchased from Fisher Chemicals, Fair Lawn, NJ. Sodium starch glycolate (Explotab®) low pH from Edward Mendell Company, Paterson, NJ. Talcum from Mallinckrodt, Inc., St. Louis, MO. Triethyl citrate from Morflex Chemical Company, Inc., Greensboro, NC.

95% Ethanol USP was purchased from Chemistry Department, Oregon State University. Water was deionized using the Milli-Q® Reagent Water System (Millipore, Bedford, MA)
Spray-Coating Procedures

Nonpareil sugar pellets 18-20 mesh (approximately 0.8 mm in diameter) were placed into a coating chamber of a fluid-bed spray coater (Niro-Aeromatic, model STREA-1, Niro-Aeromatic, Ltd.) with a Wurster column insert. The Wurster column was approximately 1 inch away from the bottom screen of the coating chamber. The sugar pellets were fluidized for 5 minutes to equilibrate with the coating temperature (40-45°C) before starting the coating process. At the end of each coating step, the coated pellets were dried in the coating chamber at 40°C for approximately 10-15 minutes.

Amphetamine salts, different polymer layers, and disintegrant were sprayed onto the sugar pellets (batch size 50-200 g) according to studied formulations in Table 5.1. Table 5.2 details compositions of the studied formulations by coating materials. Compositions of amphetamine loading solution and each coating solution/dispersion are presented in the next section. Figure 5.1 illustrates a common scheme of a non-compacted multilayered drug pellet.

All coating solutions or dispersions were continuously delivered through a feeding tube by a peristaltic pump (Rabbit Peristaltic pump, Gilson Electronics, Middleton, WI). The coating solutions or dispersions were kept stirring using a magnetic stirrer to ensure the homogeneity of the solution or dispersions. For each coating step, the coating conditions need to be carefully monitored and adjusted to maintain the optimal coating conditions. Spray coating conditions for all different coating layers are presented in Table 5.3. After each coating step, pellets were sieved to remove agglomerated and fine particles before proceeding to the next steps.
Figure 5.1 Common scheme of non-compacted multi-layered pellets
<table>
<thead>
<tr>
<th>Formulations</th>
<th>Compositions of Coated Pellet Formulations* (Drug Layer = the Innermost Layer)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug Layer</td>
</tr>
<tr>
<td>A</td>
<td>AMP1</td>
</tr>
<tr>
<td>B</td>
<td>AMP1</td>
</tr>
<tr>
<td>C</td>
<td>AMP1</td>
</tr>
<tr>
<td>D</td>
<td>AMP1</td>
</tr>
<tr>
<td>E</td>
<td>AMP1</td>
</tr>
<tr>
<td>F</td>
<td>AMP2</td>
</tr>
<tr>
<td>G</td>
<td>AMP2</td>
</tr>
<tr>
<td>H</td>
<td>AMP2</td>
</tr>
<tr>
<td>I</td>
<td>AMP2</td>
</tr>
<tr>
<td>J</td>
<td>AMP2</td>
</tr>
<tr>
<td>K</td>
<td>AMP2</td>
</tr>
<tr>
<td>L</td>
<td>AMP2</td>
</tr>
</tbody>
</table>

* Details of each coating layer are presented in the ensuing “Compositions and Preparations of Coating Solution/Dispersion” section.
* Indicates dry-blending of pellets with disintegrant mix (lactose and Explotab®) before compressing into a tablet.
AMPH amphetamine loading solution 1; AMPH2 amphetamine loading solution 2; EUED Eudragit® L 30 D-55; PEOP Polyox® N-80; EP Eudragit® with pectin; AQP1 Aquacoat® with pectin (12.5:1); AQP2 Aquacoat® with pectin (6:1); DIS1 Lactose with Explotab® (2:1); DIS2 Lactose with Explotab® (4:1).
Table 5.2: Compositions of Coated Pellet Formulations by Coating Materials

<table>
<thead>
<tr>
<th>Ingredients (Solid Composition)</th>
<th>Formulations (% of Total Coating Materials)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eudragit® L 30 D-55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>A 14.8 B 17.3 C 18.6 D 12.5 E 15.7 F 5.8 G 5.5 H 5.1 I 3.2 J 5.5 K 13.6 L 15.7</td>
</tr>
<tr>
<td>Polyox&lt;sup&gt;®&lt;/sup&gt; N-80</td>
<td>A 2.2 B 1.9 C 1.8 - - - - - - - - 2.1 -</td>
</tr>
<tr>
<td>Pectin</td>
<td>- - - - - - 0.74 0.82 1.2 - 0.82 0.53 -</td>
</tr>
<tr>
<td>Aquacoat® ECD-30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>- - - - - - 1.7 3.5 - 1.7 3.5 -</td>
</tr>
<tr>
<td>Lactose</td>
<td>49.7 47.6 46.4 54.2 50.9 60.8 71.9 70.6 76.2 71.9 48.4 51.0</td>
</tr>
<tr>
<td>Explotab&lt;sup&gt;®&lt;/sup&gt;</td>
<td>24.8 23.8 23.2 27.1 25.5 30.4 18.0 17.7 19.0 18.0 24.2 25.5</td>
</tr>
<tr>
<td>Talcum</td>
<td>7.4 8.6 9.3 6.25 7.9 2.2 2.1 2.0 1.6 2.1 6.8 7.9</td>
</tr>
<tr>
<td>HPMC E5</td>
<td>0.96 0.83 0.76 - - - - - - - 0.88 -</td>
</tr>
</tbody>
</table>

<sup>a</sup> Eudragit® L 30 D-55 suspension contains 30% polymer solid.

<sup>b</sup> Aquacoat® ECD-30 suspension contains 30% total solid (27% ethylcellulose solid).
Table 5.3 Coating Conditions for the Various Layers

<table>
<thead>
<tr>
<th>Coating Layer</th>
<th>Approximate Batch Size (g)</th>
<th>Outlet Air Temperature (°C)</th>
<th>Nozzle Diameter (mm)</th>
<th>Atomizing Air Pressure (psi)</th>
<th>Fluid Application Rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP1</td>
<td>100-200</td>
<td>45</td>
<td>0.8</td>
<td>20</td>
<td>1.7-2.0</td>
</tr>
<tr>
<td>AMP2</td>
<td>100-200</td>
<td>45</td>
<td>0.8</td>
<td>22</td>
<td>2.0-2.2</td>
</tr>
<tr>
<td>EUD</td>
<td>50-100</td>
<td>40</td>
<td>0.8</td>
<td>18</td>
<td>2.0-2.9</td>
</tr>
<tr>
<td>PEO</td>
<td>50</td>
<td>45</td>
<td>0.8</td>
<td>20-22</td>
<td>0.9-1.9</td>
</tr>
<tr>
<td>EP</td>
<td>50</td>
<td>40</td>
<td>1.0</td>
<td>22</td>
<td>2.6</td>
</tr>
<tr>
<td>AQP1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50</td>
<td>40</td>
<td>0.8</td>
<td>18</td>
<td>2.0</td>
</tr>
<tr>
<td>AQP2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50</td>
<td>40</td>
<td>0.8</td>
<td>18</td>
<td>1.5-2.0</td>
</tr>
<tr>
<td>DIS1</td>
<td>50</td>
<td>45</td>
<td>1.0</td>
<td>24</td>
<td>2.0</td>
</tr>
<tr>
<td>DIS2</td>
<td>50</td>
<td>45</td>
<td>1.0</td>
<td>24</td>
<td>2.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Coated pellets were dried in a coating chamber at 60°C for 20 minutes.

AMP1 amphetamine loading solution 1; AMP2 amphetamine loading solution 2; EUD Eudragit® L 30 D-55; PEO Polyox® N-80; EP Eudragit® with pectin; AQP1 Aquacoat® with pectin (12.5:1); AQP2 Aquacoat® with pectin (6:1); DIS1 Lactose with Explotab® (2:1); DIS2 Lactose with Explotab® (4:1).
Compositions and Preparations of Coating Solution/Dispersion

Amphetamine loading solution 1 (AMP1)

Amphetamine salts 5.0 g

(Amphetamine sulfate 1.25 g
Amphetamine aspartate 1.25 g
Dextroamphetamine sulfate 1.25 g
Dextroamphetamine saccharate 1.25 g)

Polyvinylpyrrolidone (MW. 40,000) K-30 2.0 g

Hydroxypropyl cellulose (HPC) EXF 1.0 g

Deionized water 100.0 ml

Accurately weighed HPC EXF was dispersed in 50 ml of hot deionized water. Cool deionized water was added to the well-dispersed HPC and the solution was stirred until clear. Polyvinylpyrrolidone K-30 was then added and well mixed. Finally, amphetamine salts were added to the solution and stirred until dissolved.

Amphetamine loading solution 2 (AMP2)

Amphetamine salts 8.0 g

(Amphetamine sulfate 2.0 g
Amphetamine aspartate 2.0 g
Dextroamphetamine sulfate 2.0 g
Dextroamphetamine saccharate 2.0 g)

Polyvinylpyrrolidone (MW. 40,000) K-30 16.0 g

Deionized water 100.0 ml
Polyvinylpyrrolidone K-30 was accurately weighed and dispersed in 100 ml of deionized water. Amphetamine salts were added to the solution and stirred until dissolved.

**Eudragit® L 30 D-55 dispersion (EUD)**

- Eudragit® L 30 D-55: 50.0 g
- Triethyl citrate: 3.75 g
- Talcum: 7.5 g
- Deionized water: 50.0 ml

Eudragit® L 30 D-55 was accurately weighed into a beaker. Triethyl citrate was added to Eudragit® suspension and gently mixed. Talcum was dispersed in deionized water. The talcum dispersion was then added into Eudragit® mixture and gently mixed. This mixture must be kept gently stirring.

**Polyox® N-80 dispersion (PEO)**

- Polyox® N-80: 3.5 g
- Hydroxypropyl methylcellulose (HPMC) E5: 1.5 g
- Deionized water: 15.0 ml
- 95% Ethanol: 135.0 ml

Accurately weighed HPMC E5 was dispersed in 15 ml hot deionized water. The HPMC dispersion was stirred (without heating) until well dispersed. Ethanol was
added to the well-dispersed HPMC and the solution was stirred until clear. Polyox® N-80 was then very slowly added and well mixed; if necessary, the solution can be warmed using very low heat. Allow the solution to be stirred for approximately one hour before use.

**Eudragit® with pectin dispersion (EP)**

- Eudragit® L 30 D-55: 50.0 g
- Pectin: 4.0 g
- Triethyl citrate: 1.5 g
- Talcum: 3.75 g
- Deionized water: 260.0 ml

Eudragit® L 30 D-55 was accurately weighed into a beaker. Triethyl citrate was then added and gently mixed before approximately 200 ml of deionized water was added. Dry powder of pectin was slowly dispersed in the Eudragit® L 30 D-55 suspension; the dispersion was kept stirring for approximately an hour. In a separate beaker, talcum was dispersed in the remaining amount of deionized water, and then added to the Eudragit® and pectin mixture and gently mixed.

**Aquacoat® with pectin (12.5:1) dispersion (AQP1)**

- Aquacoat® ECD-30: 12.9 g
- 1% Pectin solution: 28.0 g
- Dibutyl sebacate: 0.84 g
Aquacoat® ECD-30 was accurately weighed into a beaker. Dibutyl sebacate was added to Aquacoat® suspension and well mixed. Pectin solution (1% w/w) was added to the Aquacoat® suspension and gently mixed.

Aquacoat® with pectin (6:1) dispersion (AQP2)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquacoat® ECD-30</td>
<td>12.2 g</td>
</tr>
<tr>
<td>2% Pectin solution</td>
<td>27.4 g</td>
</tr>
<tr>
<td>Dibutyl sebacate</td>
<td>0.80 g</td>
</tr>
</tbody>
</table>

Aquacoat® ECD-30 was accurately weighed into a beaker. Dibutyl sebacate was added to Aquacoat® suspension and well mixed. Pectin solution (2% w/w) was added to the Aquacoat® suspension and gently mixed.

Lactose with Explotab® (2:1) dispersion (DIS1)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>33.3 g</td>
</tr>
<tr>
<td>Explotab®</td>
<td>16.7 g</td>
</tr>
<tr>
<td>Deionized water</td>
<td>250.0 ml</td>
</tr>
</tbody>
</table>

Accurately weighed lactose was dissolved in deionized water; if necessary, low heat may be used to facilitate the dissolution of lactose. After lactose solution cooled, Explotab® was dispersed in the solution and kept stirring.
Lactose with Explotab® (4:1) dispersion (DIS2)

Lactose 40.0 g
Explotab® 10.0 g
Deionized water 220.0 ml

Accurately weighed lactose was dissolved in deionized water; if necessary, low heat may be used to facilitate the dissolution of lactose. After lactose solution cooled, Explotab® was dispersed in the solution and kept stirring.

Drug Pellet Compaction

Tablets were compressed using a single-punch tablet machine (Carver hydraulic press, Fred S. Carver Inc. Hydraulic Equipment, Summit, NJ) fitted with round-shaped punch and die (approximately 0.375 inch in diameter). Coated pellets were compressed at a range of compaction pressure from 500 to 2000 lbs.

Amphetamine Assay

Standard Curves of Amphetamine

An exact and equal amount (12.5 mg) of each amphetamine salt was weighed and transferred to the same 100-ml volumetric flask. The sample was dissolved in either simulated gastric fluid (pH 1.4) or phosphate buffer (pH 6.0) and adjusted to final volume. This stock solution contained equal amounts of each amphetamine salt and had total amphetamine salts concentration of 500 μg/ml. A series of standard solutions with a concentration of 5-200 μg/ml (5-100 μg/ml in
simulated gastric fluid) was prepared from the stock solution by dilution. UV absorbance of standard solutions was measured by UV spectrophotometer at 257 nm. Amphetamine standard curves are presented in Figures 5.2 and 5.3.

Drug Content Assay

Drug content assay was performed in triplicate. An exact amount (100-150 mg) of coated pellets was weighed and transferred to 100-ml volumetric flasks. Phosphate buffer was used to dissolve drug pellets. Either a stirring bar or a sonicator bath was used to facilitate the disintegration and dissolution. After drug pellets were completely disintegrated, the samples were centrifuged at 3000 rpm for 10-20 minutes. Supernatant was then collected and measured by UV spectrophotometer at 257 nm. The amount of amphetamine contained in each formulation was determined using an appropriate standard curve.

In Vitro Dissolution Testing of Formulations

In vitro drug release profiles of studied formulations were obtained using the USP XXV dissolution apparatus II, paddle stirring method (VK 7000, Vankel Industries, Inc., Cary, NC). Dissolution was studied at a paddle rotation speed of 100 rpm and temperature of dissolution bath was maintained at 37.5°C. Dissolution testing of amphetamine formulations was performed in triplicate.

Three different forms of studied formulations were prepared for dissolution testing: 1) intact pellets, 2) tablet, and 3) crushed tablet using a commercial tablet crusher (EZ Swallow, American Medical Industries, Highland Park, IL). Studied formulations were placed into dissolution vessels containing 375 ml of simulated gastric fluid. Dissolution testing was run in simulated gastric fluid for 2 hours. At the end of the 2-hour period, the dissolution medium was adjusted to pH 6.0 by
adding approximately 50 ml of 0.2 M tribasic sodium phosphate solution and sufficient amount of deionized water to make a final volume of 500 ml.

Five (5) ml of samples were manually collected without medium replacement at 0.25, 0.5, 1, 2, 2.25, 2.5, 2.75, 3, 3.5, and 4 hours. Due to availability of prior information on likelihood of dissolution profile of some studied formulations, slight adjustment was made to the sample collection schedule of those formulations in order to achieve the most informative dissolution profile. The samples were centrifuged at 3000 rpm for 10-20 minutes. Supernatant was then collected and measured by UV spectrophotometer at 257 nm. The amount of amphetamine released was determined using an appropriate standard curve.

Average drug releases and their standard deviations were calculated from three replications in all dissolution experiments. Amphetamine dissolution profiles are presented as percent drug release versus time curves.
Figure 5.2 Standard curve of amphetamine in simulated gastric fluid (UV wavelength at 257 nm)
Figure 5.3 Standard curve of amphetamine in phosphate buffer pH 6.0 (UV wavelength at 257 nm)
RESULTS AND DISCUSSION

Different enteric-coated pellets in crushable tablet formulations were prepared according to details of formulations in Tables 5.1 and 5.2. Disintegrant layer was spray-coated onto pellets as a final step for formulations A, B, C, D, E, J K, and L. Tablets were formed by direct compression of disintegrant-coated pellets without additional excipients. Disintegrant was not spray-coated for formulations F, G, H, and I. For these formulations, tablets were formed by direct compression of dry-blend of pellets and disintegrant mix (lactose and Explotab®).

All studied formulations consisted of an innermost amphetamine layer, enteric-coating layer(s), and/or hydrophilic polymer layer(s). Two different amphetamine-loading solutions were used in the study. In the AMP2 layer, high amount of PVP K-30 was used in amphetamine-loading solution in order to study its effects on drug release profile from compressed enteric-coated pellets.

Dissolution Studies

Formulation A (AMP1 + 20%EUD + 5%PEO + 7.5%EUD + 100%DIS1) was compressed into tablets at 500 and 1500 lbs compression pressure, and its dissolution profiles presented in Figures 5.4 and 5.5, respectively. Crushed tablet released amphetamine approximately 35% at 2 hours in simulated gastric fluid regardless of compression pressure. In the other words, more than 60% of amphetamine was protected by the formulation, even though the pellets were compressed into tablets and the tablets were then crushed. Uncrushed tablet compressed at 1500 lbs, however, did not completely disintegrate within one hour.
causing drug release to be less (20% release at 2 hours) when compared to
uncrushed tablet compressed at 500 lbs (35% release at 2 hours). Non-compacted
pellets released the drug approximately 10% at 2 hours in simulated gastric fluid.
When dissolution medium pH was adjusted to 6.0, all samples completely released
the drug within the first hour (hour 3 from the beginning of the dissolution testing),
with the tablet and the crushed tablet achieving complete drug release slightly faster
than the non-compacted pellets (Fig. 5.5).

Dissolution profiles of formulation B (AMP1 + 20%EUD + 5%PEO +
15%EUD + 100%DIS1) are presented in Figure 5.6. Both uncrushed and crushed
tablet released amphetamine approximately 60% at 2 hours in simulated gastric
fluid. Non-compacted pellets released the drug approximately 5% at 2 hours in
simulated gastric fluid. Drug releases after adjusting dissolution medium pH to 6.0
were similar to formulation A.

Formulation C (AMP1 + 20%EUD + 5%PEO + 20%EUD + 100%DIS1)
was compressed into tablets at 500 and 1500 lbs compression pressure, and
dissolution profiles are presented in Figures 5.7 and 5.8, respectively. Both
uncrushed and crushed tablet released amphetamine between 60-70% at 2 hours in
simulated gastric fluid. Non-compacted pellets released the drug approximately 5%
at 2 hours in simulated gastric fluid. Drug releases after adjusting dissolution
medium pH to 6.0 were similar to formulations A and B.

Formulations A, B, and C are very similar in compositions of polymer
layers. The only difference is in the amount of Eudragit® in the outer Eudragit®
layer (EUD), where there is 7.5%, 15%, and 20% Eudragit®, respectively.
Formulations B and C have higher amount of Eudragit® than does formulation A,
but with the same amount of Polyox®; therefore, formulations B and C theoretically
should provide better or equal protection against compressing and crushing. This
does not seem to be the case here. When formulations B and C are compared to
formulations D (AMP1 + 20%EUD + 100%DIS1, Figure 5.9) and E (AMP1 + 20%EUD + 7.5%EUD + 100%DIS1, Figure 5.10), which serve as standard enteric-coated formulations (without Polyox®), they provide no better protection. It is speculated that the amount of Polyox® (5%) used in these formulations is insufficient for protecting the drug from being released in gastric fluid from either compressed tablets or crushed tablets. Increasing Polyox® in these formulations may increase protection to the damaged coating, thus decrease amphetamine release in simulated gastric fluid, and improve consistency of the formulations.

Dissolution profiles of formulation F (AMP2 + 9.5%EP + 7.5%EUD + 200%DIS1*) are presented in Figure 5.11. Tablet was made by direct compression of dry-blend of pellets and disintegrant mix (lactose and Explotab®) at 1500 lbs compression pressure. Uncrushed tablet and crushed tablet released amphetamine approximately 30% and 40%, respectively, at 2 hours in simulated gastric fluid. Non-compacted pellets released the drug approximately 5% at 2 hours in simulated gastric fluid. When dissolution medium pH was adjusted to 6.0, all samples completely released the drug within 30 minutes (Hour 2.5 from the beginning of the dissolution testing).

Dissolution profiles of formulation G (AMP2 + 9.5%EP + 7.5%EUD + 5%AQP1 + 200%DIS2*) are presented in Figure 5.12. Tablet was made by direct compression of dry-blend of pellets and disintegrant mix (lactose and Explotab®) at 2000 lbs compression pressure. Both uncrushed tablet and crushed tablet released amphetamine approximately 25-30% at 2 hours in simulated gastric fluid. Non-compacted pellets released the drug approximately 5% at 2 hours in simulated gastric fluid. When dissolution medium pH was adjusted to 6.0, uncrushed and crushed tablet completely released the drug within 30 minutes, while non-compacted pellets completely released the drug at 2 hours in pH 6.0 (Hour 4 from the beginning of the dissolution testing). The slow dissolution profile for non-
compacted pellets resulted from ethylcellulose (a pH-independent, water-insoluble polymer) contained in Aquacoat® with pectin (AQP1) layer.

Dissolution profiles of formulation H (AMP2 + 9.5%EP + 7.5%EUD + 12%AQP2 + 200%DIS2*) are presented in Figure 5.13. Tablet was made by direct compression of dry-blend of pellets and disintegrant mix (lactose and Explotab®) at 2000 lbs compression pressure. Formulations H and G have similar dissolution profiles.

Formulation J (AMP2 + 9.5%EP + 7.5%EUD + 5%AQP1 + 200%DIS2) was compressed into tablets at 1000 lbs compression pressure, and its dissolution profiles are presented in Figure 5.15. The formulation was unable to protect the drug release against compressing or crushing. Both uncrushed tablet and crushed tablet released amphetamine about 80% in 15 minutes and more than 90% at 2 hours in simulated gastric fluid.

When tablets are formed by direct compression of dry-blend of pellets and disintegrant mix, there is less impact on pellets than from direct compression of disintegrant-coated pellets. When pellets are mixed with dry powder of disintegrant, disintegrant can fill the void volume between pellets forming a cushion that prevents deformation of pellets after being compressed. Unlike compressing of dry blend, compressing of disintegrant-coated pellets is likely to cause more deformation of pellets, subsequently more damaged polymer layers, to some extent depending on characteristics and amount of disintegrants.

Formulations G and H, which contains Eudragit® with pectin and Aquacoat® with pectin, can only provide some protection against small compaction force causing by direct compression of dry-blend of pellets and disintegrant mix. The protective ability of Eudragit® with pectin and Aquacoat® with pectin are shown by comparing formulations G and H (Figures 5.12 and 5.13) with formulation I (AMP2 + 7.5%EUD + 200%DIS2*, Figure 5.14). Formulation I
contains neither Eudragit® with pectin nor Aquacoat® with pectin, and was unable to withstand even small compaction force causing by direct compression of dry-blend of pellets and disintegrant mix. The protective ability of Eudragit® with pectin and Aquacoat® with pectin may still be useful when used in conjunction with disintegrants that have better cushioning effects than a combination of lactose and Explotab®.

Formulation K (AMP2 + 20%EUD + 5% PEO + 7.5%EUD + 7%AQP2 + 100%DIS1) was compressed into tablets at 500 lbs compression pressure, and its dissolution profiles were presented in Figure 5.16. Good protection against compaction and crushing was obtained from this formulation. Both uncrushed tablet and crushed tablet released amphetamine approximately 25% at 2 hours in simulated gastric fluid. Non-compacted pellets released the drug less than 5% at 2 hours in simulated gastric fluid. When dissolution medium pH was adjusted to 6.0, uncrushed and crushed tablet completely released the drug within 45 minutes, while non-compacted pellets had different release pattern. Non-compacted pellets of formulation K released the drug only 40% after 3 hours in pH 6.0 (Hour 5 from the beginning of the dissolution testing). When a similar formulation without Polyox® and Aquacoat® with pectin was made (formulation L) and dissolution profiles were obtained, the formulation lost the protective properties against compaction and crushing. Formulation L (AMP2 + 20%EUD + 7.5%EUD + 100%DIS1, Figure 5.17) released the drug approximately 70-80% at 2 hours in simulated gastric fluid when compressed or crushed.

Compositions of amphetamine-loading solution do not appear to have significant effects on drug release from the pellets. Nevertheless, when dissolution profiles from formulation E (AMP1 + 20%EUD + 7.5%EUD + 100%DIS1) is compared to those from formulation L (AMP2 + 20%EUD + 7.5%EUD + 100%DIS1), amphetamine release from formulation E (50-60%, Figure 5.10) is less
than the release from formulation L (70-80%, Figure 5.17) at 2 hours in simulated gastric fluid. It appears that high amount of PVP K-30 used in amphetamine layer has no effect on sustaining drug release from formulations.
Figure 5.4 Effects of compaction (500 lbs) and crushing on drug release from formulation A (AMP1 + 20%EUD + 5%PEO + 7.5%EUD + 100%DIS1)
Figure 5.5 Effects of compaction (1500 lbs) and crushing on drug release from formulation A (AMP1 + 20%EUD + 5%PEO + 7.5%EUD + 100%DIS1)
Figure 5.6 Effects of compaction (1500 lbs) and crushing on drug release from formulation B (AMP1 + 20%EUD + 5%PEO + 15%EUD + 100%DIS1)
Figure 5.7 Effects of compaction (500 lbs) and crushing on drug release from formulation C (AMP1 + 20%EUD + 5%PEO + 20%EUD + 100%DIS1)
Figure 5.8 Effects of compaction (1500 lbs) and crushing on drug release from formulation C (AMP1 + 20%EUD + 5%PEO + 20%EUD + 100%DIS1)
Figure 5.9 Effects of compaction (1500 lbs) and crushing on drug release from formulation D (AMP1 + 20%EUD + 100%DIS1)
Figure 5.10  Effects of compaction (1500 lbs) and crushing on drug release from formulation E (AMP1 + 20%EUD + 7.5%EUD + 100%DIS1)
Figure 5.11  Effects of compaction (1500 lbs) and crushing on drug release from formulation F (AMP2 + 9.5%EP + 7.5%EUD + 200%DIS1*)
Figure 5.12  Effects of compaction (2000 lbs) and crushing on drug release from formulation G (AMP2 + 9.5%EP + 7.5%EUD + 5%AQP1 + 200%DIS2*)
Figure 5.13  Effects of compaction (2000 lbs) and crushing on drug release from formulation H (AMP2 + 9.5%EP + 7.5%EUD + 12%AQP2 + 200%DIS2*)
Figure 5.14  Effects of compaction (2000 lbs) and crushing on drug release from formulation I (AMP2 + 7.5%EUD + 200%DIS2*)
Figure 5.15  Effects of compaction (1000 lbs) and crushing on drug release from formulation J (AMP2 + 9.5%EP + 7.5%EUD + 5%AQP1 + 200%DIS2)
Figure 5.16  Effects of compaction (500 lbs) and crushing on drug release from formulation K (AMP2 + 20%EUD + 5%PEO + 7.5%EUD + 7%AQP2 + 100%DIS1)
Figure 5.17  Effects of compaction (500 lbs) and crushing on drug release from formulation L (AMP2 + 20%EUD + 7.5%EUD + 100%DIS1)
CONCLUSIONS

Crushable enteric-coated formulations of amphetamine were prepared using spray-coating technique. Drug-loading solution, polymer solutions/dispersions, and disintegrant dispersion were spray-coated on to sugar core pellets, forming multi-layered drug pellets. Multi-layered pellets can be easily produced and can be directly compressed into tablets without additional tableting excipients due to their good flowability.

All studied crushable enteric-coated formulations consisted of an innermost amphetamine layer, enteric-coating layer(s), and hydrophilic polymer layer(s). Enteric-coating layer and hydrophilic polymer layer are crucial for crushable enteric-coated formulations as described in U.S. Patent 5,766,623 (4). Hydrophilic polymer layer provide abilities to self-seal crack caused to the coating film by compacting and crushing of the pellets, thus prevent the formulations from losing controlled-release properties.

Polyox® N-80 at studied amount (5%) was likely to be the borderline amount required for protecting the formulations against compaction and crushing. The desired sealant effect may be achieved by increasing the amount of Polyox® in the formulation.

Eudragit® with pectin and Aquacoat® with pectin provide some protection against small compaction force causing by direct compression of dry-blend of pellets and disintegrant mix. They may be useful when used in conjunction with disintegrants that have better cushioning effects than a combination of lactose and Explotab®.
Combination of Polyox® and Aquacoat® with pectin was shown to have good protective properties against compaction and crushing. However, they do not produce similar drug release pattern to non-compacted pellets when dissolution medium was adjusted to pH 6.0. Such difference will not be a concern if the formulation is to be compressed into tablets instead of filling in capsules, since the drug release pattern from tablet is similar to crushed tablet.

Although none of the studied formulations were able to fulfill USP XXV requirements regarding amount released allowed in acidic medium at 2 hours, which was limited to 10% or less, results from the present research were very promising for future development of crushable/chewable enteric-coated formulations.
REFERENCES


CHAPTER 6

DEVELOPMENT OF LEAKY ENTERIC-COATED PELLETS FORMULATIONS

Prapoch Watanalumlerd and James W. Ayres
ABSTRACT

"Leaky" enteric-coated pellets formulations of three model drugs known to have an absorption window (riboflavin-5-phosphate, ranitidine hydrochloride, and hydrochlorothiazide) were produced by spray-coating. Leaky enteric coats were formulated using a commonly used enteric polymer, Eudragit® L 30 D-55, combined with soluble compound/polymer, lactose or HPMC. The rate of drug release from the formulations in simulated gastric fluid was controlled by modifying the leaky enteric coat content and/or varying coating amount. All leaky enteric-coated formulations studied completely released the drugs within 30 minutes after changing dissolution medium to phosphate buffer pH 6.0.

Predictions of plasma concentration-time profiles of model drugs from leaky enteric-coated pellets with various "leakage rates" in both fed and fasted conditions were performed using computer simulations. The simulations results are consistent with a hypothesis that leaky enteric-coated pellets formulations were able to provide sustained-input for drugs shown to have an absorption window without decreasing bioavailability and in some cases were considered with improved bioavailability of those drugs.

The present research demonstrated a new use of knowledge about gastrointestinal transit in drug formulations. It also showed that enteric polymers have uses in areas other than traditional enteric-coated formulations. The hypothesis that a leaky enteric-coated pellets formulation can increase bioavailability of drugs that have a window of absorption is still to be confirmed by in vivo studies, but is consistent with data obtained using gastric retention formulations (1-4).
INTRODUCTION

Many drugs are known to have an absorption window, for example, furosemide, hydrochlorothiazide, riboflavin, amino acids, and digoxin (5-7). This means the absorption of drugs takes place in certain regions of the gastrointestinal (GI) tract, usually the upper intestinal tract. Some drugs, such as ranitidine, have significantly limited absorption once they reach the large intestine (8). Williams et al. (8) showed that the relative bioavailability of ranitidine following cecal administration (via a nasoenteric tube) was less than 15% of that observed after administration into the stomach or jejunum.

For drugs that have an absorption window, administering the drugs as sustained-release formulations while maintaining similar bioavailability is not feasible. Typical sustained-release formulations, depending on types of formulation, may begin releasing drugs as soon as the formulations are ingested. The release continues even when the formulations are in the lower intestinal tract. This causes absorption of the drugs to become significantly reduced once the formulations have passed an absorption window region of the GI tract.

A few approaches have been studied to produce sustained-release formulations of drugs exhibiting an absorption window by retaining the formulations in the stomach or upper intestinal tract as long as possible. These approaches include the use of mucoadhesive microspheres (5), a floating system (9), and a gastroretentive dosage form (10). Sustained-release mucoadhesive microspheres of furosemide and riboflavin have been formulated and the formulations were investigated in humans (5). The relative bioavailability was 1.8 times higher for furosemide and was 2.4 times higher for riboflavin when mucoadhesive microspheres rather than when non-adhesive microspheres were
administered in fasted volunteers (5). Even though some of these approaches seemed to work well in terms of achieving sustained-absorption of the drugs, they had several crucial limitations such as scale-up challenge, and preparation involving a use of high temperature (90 degrees C).

Chapters 2 and 3 discussed how GI transit affects pharmacokinetics of drugs from enteric-coated pellets. It was shown that when administered as enteric-coated pellets, especially in the fed condition, plasma concentration profiles of drugs was similar to those from sustained-release formulations due to gradual emptying of drug pellets from the stomach. In addition, gastric emptying of drug pellets was more predictable than that of single-unit enteric-coated formulation (e.g. tablet).

The present research proposed a concept of using modified enteric-coated pellets formulation to provide a sustained release of drugs that have an absorption window and, at the same time, to improve or maintain similar bioavailability compared to an immediate-release formulation. The enteric coat was modified such that weakened enteric polymer allowed some amounts of drugs to release while the pellets were in the stomach. The modified enteric-coated pellets formulations were recognized in this research as “leaky” enteric-coated pellets formulations.

It was hypothesized that leaky enteric-coated pellets formulations were able to provide sustained-release and sustained drug input effect without decreasing bioavailability because saturation of drugs at absorption window occurred minimally or not at all. When leaky enteric-coated pellets are administered, some portion of drugs will be released in the stomach. If the stomach is one of drugs’ absorption sites, some of the drugs will be absorbed instantaneously. Then dissolved drugs will be emptied along with fluid content in the stomach into an upper intestinal tract, the duodenum, and absorbed there or in the upper jejunum. At the same time, intact pellets will slowly enter the duodenum and then quickly
disintegrate and release any remaining portion of the drugs due to already weakened enteric coat. Therefore, all of the drugs are not present at an absorption site at the same time, reducing a chance to saturate the site. Also, since the formulation disintegrates rapidly once it reaches the duodenum, it is unlikely that the formulation will pass the absorption window before it completely releases the drugs.

Riboflavin-5-phosphate, ranitidine hydrochloride, and hydrochlorothiazide were chosen as model drugs in this research. Bioavailability of immediate-release riboflavin in the fed condition was found to be 74.6%, 43.3% and 36.4% following an oral dose of 20 mg, 40 mg and 60 mg, respectively (11). Bioavailability of 100 mg immediate-release ranitidine was 51% to 58% in the fasted condition (12-14). Bioavailability of hydrochlorothiazide (25-100 mg dose) in the fasted condition ranged from 50.3 % to 78% (15-20).

The objectives of the present research are:

1. To produce leaky enteric-coated pellets formulations that release some of drugs in simulated gastric condition in a controlled fashion and instantaneously release remaining portion of drugs when transferred into simulated intestinal condition.

2. To demonstrate effects of leaky enteric-coated pellets formulations on plasma concentration-time profiles of drugs that have an absorption window using computer simulations.
MATERIALS AND METHODS

Chemicals

All chemicals used in this study were obtained from standard sources. Riboflavin-5-phosphate was purchased from Sigma Chemical Co., St. Louis, MO. Ranitidine hydrochloride was obtained from Avocado Research Chemicals Ltd., Heysham, Lancs, UK. Hydrochlorothiazide was purchased from Sigma-Aldrich Co., St. Louis, MO.

Hydroxypropyl cellulose (Klucel®) EXF was obtained from Aqualon, Wilmington, DE. Hydroxypropyl methylcellulose (HPMC) E5 was received from Teva Pharmaceuticals, PA. Lactose monohydrate was purchased from J.T. Baker Chemical Co., Phillipsburg, NJ. Methacrylic acid copolymer (Eudragit® L 30 D-55) was obtained from Röhm Tech Inc., Malden, MA. Polyvinylpyrrolidone (PVP) K-30 (MW. 40,000) from Spectrum Quantity Products, Inc., New Brunswick, NJ. Sodium chloride, Sodium phosphate tribasic, Concentrated hydrochloric acid were purchased from Fisher Chemicals, Fair Lawn, NJ. Talcum from Mallinckrodt, Inc., St. Louis, MO. Triethyl citrate from Aldrich Chemical Company, Inc., Milwaukee, WI.

95% Ethanol USP was obtained from Chemistry Department, Oregon State University. Water was deionized using the Milli-Q® Reagent Water System (Millipore, Bedford, MA)
Spray-Coating Procedures

Nonpareil sugar pellets 18-20 mesh (approximately 0.8 mm in diameter) were placed into a coating chamber of a fluid-bed spray coater (Niro-Aeromatic, model STREA-1, Niro-Aeromatic, Ltd.) with a Wurster column insert. The Wurster column was approximately 1 inch away from the bottom screen of the coating chamber. The sugar pellets were fluidized for 5 minutes to equilibrate with the coating temperature (40-45°C) before starting the coating process. At the end of each coating step, the coated pellets were dried in the coating chamber at 40°C for approximately 10-15 minutes.

Figure 6.1 illustrates a common scheme of a leaky enteric-coated pellet. Model drugs and leaky enteric-coating polymers were sprayed onto the sugar pellets (batch size 40-200 g) according to studied formulations in Tables 6.1-6.3. Detailed compositions of leaky enteric-coated layer of the studied formulations are shown in Tables 6.4-6.6. Compositions and preparations of coating solution/ dispersion for each model drug are elaborated under each corresponding section.

All coating solutions or dispersions were continuously delivered through a feeding tube by a peristaltic pump (Rabbit Peristaltic pump, Gilson Electronics, Middleton, WI). The coating solutions or dispersions were kept stirring using a magnetic stirrer to ensure homogeneity of the solution or dispersions. For each coating step, the coating conditions were carefully monitored and adjusted to maintain excellent coating conditions. After each coating step, pellets were sieved to remove agglomerated and fine particles before proceeding to the next steps.
Figure 6.1 Common scheme of non-compacted multi-layered pellets

Table 6.1 Leaky Enteric-Coated Pellets Formulations of Riboflavin-5-Phosphate

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Composition of Leaky Enteric-Coating Polymer</th>
<th>Amount of Leaky Enteric-Coating Polymer (% of Drug-Loaded Pellets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF1</td>
<td>EUD(^a)</td>
<td>5%</td>
</tr>
<tr>
<td>RF2</td>
<td>EUD with 50% lactose</td>
<td>5%</td>
</tr>
<tr>
<td>RF3</td>
<td>EUD with 65% lactose</td>
<td>5%</td>
</tr>
<tr>
<td>RF4</td>
<td>EUD with 83.5% lactose</td>
<td>5%</td>
</tr>
</tbody>
</table>

\(^a\) Eudragit\(^®\) L 30 D-55 (EUD)

\(^b\) Amount of leaky enteric-coating polymer is presented as an amount of Eudragit\(^®\) L 30 D-55 polymer solid (in leaky enteric-coat layer) coated onto drug-loaded pellets. Eudragit\(^®\) L 30 D-55 suspension contains 30% polymer solid.
Table 6.2 Leaky Enteric-Coated Pellets Formulations of Ranitidine Hydrochloride

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Composition of Leaky Enteric-Coating Polymer</th>
<th>Amount of Leaky Enteric-Coating Polymer (% of Drug-Loaded Pellets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTD1</td>
<td>EUD a with 33% lactose</td>
<td>7.5%</td>
</tr>
<tr>
<td>RTD2</td>
<td>EUD with 33% lactose</td>
<td>10%</td>
</tr>
<tr>
<td>RTD3</td>
<td>EUD with 33% lactose</td>
<td>12.5%</td>
</tr>
<tr>
<td>RTD4</td>
<td>EUD with 33% lactose</td>
<td>15%</td>
</tr>
<tr>
<td>RTD5</td>
<td>EUD with 50% lactose</td>
<td>10%</td>
</tr>
<tr>
<td>RTD6</td>
<td>EUD with 50% lactose</td>
<td>12.5%</td>
</tr>
<tr>
<td>RTD7</td>
<td>EUD with 50% lactose</td>
<td>15%</td>
</tr>
</tbody>
</table>

a Eudragit® L 30 D-55 (EUD)  
b Amount of leaky enteric-coating polymer is presented as an amount of Eudragit® L 30 D-55 polymer solid (in leaky enteric-coat layer) coated onto drug-loaded pellets. Eudragit® L 30 D-55 suspension contains 30% polymer solid.

Table 6.3 Leaky Enteric-Coated Pellets Formulations of Hydrochlorothiazide

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Composition of Leaky Enteric-Coating Polymer</th>
<th>Amount of Leaky Enteric-Coating Polymer (% of Drug-Loaded Pellets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCTZ1</td>
<td>EUD a with 20% HPMC b</td>
<td>7.5%</td>
</tr>
<tr>
<td>HCTZ2</td>
<td>EUD with 5% HPMC</td>
<td>5%</td>
</tr>
<tr>
<td>HCTZ3</td>
<td>EUD with 5% HPMC</td>
<td>7.5%</td>
</tr>
<tr>
<td>HCTZ4</td>
<td>EUD with 5% HPMC</td>
<td>10%</td>
</tr>
</tbody>
</table>

a Eudragit® L 30 D-55 (EUD)  
b Hydroxypropyl methylcellulose (HPMC) E5  
c Amount of leaky enteric-coating polymer is presented as an amount of Eudragit® L 30 D-55 polymer solid (in leaky enteric-coat layer) coated onto drug-loaded pellets. Eudragit® L 30 D-55 suspension contains 30% polymer solid.
Table 6.4 Solid Compositions of Leaky Enteric-Coated Layer of Riboflavin-5-Phosphate Formulations

<table>
<thead>
<tr>
<th>Ingredients (Solid Composition)</th>
<th>Formulations (% of Total Coating Materials)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RF1</td>
</tr>
<tr>
<td>Eudragit® L 30 D-55</td>
<td>66.7</td>
</tr>
<tr>
<td>Talcum</td>
<td>33.3</td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 6.5 Solid Compositions of Leaky Enteric-Coated Layer of Ranitidine Hydrochloride Formulations

<table>
<thead>
<tr>
<th>Ingredients (Solid Composition)</th>
<th>Formulations (% of Total Coating Materials)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RTD1</td>
</tr>
<tr>
<td>Eudragit® L 30 D-55</td>
<td>54.5</td>
</tr>
<tr>
<td>Talcum</td>
<td>27.3</td>
</tr>
<tr>
<td>Lactose</td>
<td>18.2</td>
</tr>
</tbody>
</table>

Table 6.6 Solid Compositions of Leaky Enteric-Coated Layer of Hydrochlorothiazide Formulations

<table>
<thead>
<tr>
<th>Ingredients (Solid Composition)</th>
<th>Formulations (% of Total Coating Materials)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCTZ1</td>
</tr>
<tr>
<td>Eudragit® L 30 D-55</td>
<td>58.8</td>
</tr>
<tr>
<td>Talcum</td>
<td>29.4</td>
</tr>
<tr>
<td>HPMC E5</td>
<td>11.8</td>
</tr>
</tbody>
</table>
Compositions and Preparations of Coating Solution/Dispersion

Compositions and preparations of drug-loading solution, coating solution/dispersion are described as follows. Spray-coating conditions for drug-loading solution and coating polymers are presented in Table 6.7.

**Riboflavin-5-Phosphate**

Riboflavin loading solution

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riboflavin-5-phosphate</td>
<td>7.5 g</td>
</tr>
<tr>
<td>PVP K-30</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Hydroxypropyl cellulose (HPC) EXF</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Deionized water</td>
<td>250.0 ml</td>
</tr>
</tbody>
</table>

Accurately weighed HPC EXF was dispersed in 50 ml of hot deionized water. Cool deionized water was added to the well-dispersed HPC and the solution was stirred until clear. PVP K-30 was then added and well mixed. Finally, riboflavin was added to the solution and stirred until dissolved. Loading solution was kept protected from light throughout this process.

**Eudragit® L 30 D-55 dispersion (EUD)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eudragit® L 30 D-55</td>
<td>50.0 g</td>
</tr>
<tr>
<td>Triethyl citrate</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Talcum</td>
<td>7.5 g</td>
</tr>
<tr>
<td>Deionized water</td>
<td>50.0 ml</td>
</tr>
</tbody>
</table>
Eudragit® L 30 D-55 was accurately weighed into a beaker. Triethyl citrate was added to Eudragit® suspension and gently mixed. Talcum was dispersed in deionized water. The talcum dispersion was then added into Eudragit® mixture and gently mixed. This mixture was kept gently stirring.

Eudragit® L 30 D-55 with 50% lactose dispersion

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eudragit® L 30 D-55</td>
<td>50.0 g</td>
</tr>
<tr>
<td>Triethyl citrate</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Talcum</td>
<td>7.5 g</td>
</tr>
<tr>
<td>Lactose</td>
<td>7.5 a g</td>
</tr>
<tr>
<td>Deionized water</td>
<td>125.0 b ml</td>
</tr>
</tbody>
</table>

Dissolved accurately weighed lactose in 75 ml of deionized water (solution may be warmed to facilitate the dissolution). Talcum was dispersed in the remaining deionized water. Talcum dispersion was added to lactose solution and kept stirring. Eudragit® L 30 D-55 was accurately weighed into a beaker. Triethyl citrate was added to Eudragit® suspension and gently mixed. The lactose and talcum dispersion was then added into Eudragit® mixture and gently mixed. This mixture was kept gently stirring.

Note:  

- Amount of lactose used in studied formulations was calculated as percentage of Eudragit® polymer solid (Eudragit® polymer suspension contains 30% polymer solid).
- Volume of deionized water varied as needed to sufficiently dissolve lactose (generally, one part of lactose can be comfortably dissolved in 10 parts of water).
**Ranitidine Hydrochloride**

**Ranitidine loading solution**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranitidine hydrochloride</td>
<td>7.5 g</td>
</tr>
<tr>
<td>PVP K-30</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Hydroxypropyl cellulose (HPC) EXF</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Deionized water</td>
<td>60.0 ml</td>
</tr>
</tbody>
</table>

Accurately weighed HPC EXF was dispersed in 30 ml of hot deionized water. Cool deionized water was added to the well-dispersed HPC and the solution was stirred until clear. PVP K-30 was then added and well mixed. Finally, ranitidine was added to the solution and stirred until dissolved.

Note: a Ranitidine HCl is hygroscopic; therefore, ranitidine-loaded pellets should be kept in a tightly closed container.

**Eudragit® L 30 D-55 with 33% lactose dispersion**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eudragit® L 30 D-55</td>
<td>50.0 g</td>
</tr>
<tr>
<td>Triethyl citrate</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Talcum</td>
<td>7.5 g</td>
</tr>
<tr>
<td>Lactose</td>
<td>5.0 a g</td>
</tr>
<tr>
<td>Deionized water</td>
<td>100.0 bml</td>
</tr>
</tbody>
</table>

Dissolved accurately weighed lactose in 50 ml of deionized water (solution may be warmed to facilitate the dissolution). Talcum was dispersed in the remaining deionized water. Talcum dispersion was added to lactose solution and kept stirring. Eudragit® L 30 D-55 was accurately weighed into a beaker. Triethyl citrate was
added to Eudragit® suspension and gently mixed. The lactose and talcum dispersion was then added into Eudragit® mixture and gently mixed. This mixture must be kept gently stirring.

Note:  
\(a\) Amount of lactose used in studied formulations was calculated as percentage of Eudragit® polymer solid (Eudragit® polymer suspension contains 30% polymer solid).

\(b\) Volume of deionized water varied as needed to sufficiently dissolve lactose (generally, one part of lactose can be comfortably dissolved in 10 parts of water).

### Hydrochlorothiazide

**Hydrochlorothiazide loading solution**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrochlorothiazide</td>
<td>5.0 g</td>
</tr>
<tr>
<td>PVP K-30</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Deionized water</td>
<td>30.0 ml</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>500.0 ml</td>
</tr>
</tbody>
</table>

Accurately weighed hydrochlorothiazide was dissolved in 500 ml of ethanol (solution may be warmed to facilitate the dissolution). PVP K-30 was dispersed in 30 ml of deionized water before being added to hydrochlorothiazide solution and well mixed.
Accurately weighed HPMC E5 was dispersed in approximately 15 ml of hot deionized water. Fifteen (15) ml of cool deionized water was added to the well-dispersed HPMC and the solution was stirred until clear. Talcum was dispersed in the remaining deionized water. Talcum dispersion was added to HPMC solution and kept stirring. Eudragit® L 30 D-55 was accurately weighed into a beaker. Triethyl citrate was added to Eudragit® suspension and gently mixed. The HPMC and talcum dispersion was then added into Eudragit® mixture and gently mixed. This mixture was kept gently stirring.

Note: Amount of HPMC used in studied formulations was calculated as percentage of Eudragit® polymer solid (Eudragit® polymer suspension contains 30% polymer solid).
### Table 6.7 Coating Conditions for Leaky Enteric-Coated Formulations

<table>
<thead>
<tr>
<th>Coating Layer</th>
<th>Approximate Batch Size (g)</th>
<th>Outlet Air Temperature (°C)</th>
<th>Nozzle Diameter (mm)</th>
<th>Atomizing Air Pressure (psi)</th>
<th>Fluid Application Rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF Loading Solution</td>
<td>100</td>
<td>48</td>
<td>0.8</td>
<td>22</td>
<td>2.2</td>
</tr>
<tr>
<td>RTD Loading Solution</td>
<td>100-200</td>
<td>45</td>
<td>0.8</td>
<td>18</td>
<td>0.7-0.8</td>
</tr>
<tr>
<td>HCTZ Loading Solution</td>
<td>200</td>
<td>45</td>
<td>0.8</td>
<td>18</td>
<td>6.0-7.0</td>
</tr>
<tr>
<td>EUD</td>
<td>45</td>
<td>40</td>
<td>0.8</td>
<td>18</td>
<td>2.9</td>
</tr>
<tr>
<td>EUD with Lactose</td>
<td>40-50</td>
<td>40</td>
<td>0.8</td>
<td>18</td>
<td>1.2-3.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>EUD with HPMC</td>
<td>60-70</td>
<td>40</td>
<td>0.8</td>
<td>18</td>
<td>1.5-2.0</td>
</tr>
</tbody>
</table>

**RF** (Riboflavin-5-phosphate); **RTD** (Ranitidine hydrochloride); **HCTZ** (Hydrochlorothiazide); **EUD** (Eudragit®); **HPMC** (Hydroxypropyl methylcellulose)

<sup>a</sup> For RTD formulations, fluid application rate for leaky enteric-coat layer was in a lower range (1.2-1.8 ml/min).
Methods of Drug Assay

Riboflavin-5-Phosphate Assay

Standard Curves of Riboflavin

An exact amount (50.0 mg) of riboflavin-5-phosphate was weighed and transferred to a 100-ml volumetric flask. The sample was dissolved in simulated gastric fluid (pH 1.4) and adjusted to the final volume. This stock solution had riboflavin-5-phosphate concentration of 500 μg/ml. A series of standard solutions with a concentration of 5-100 μg/ml was prepared from the stock solution by dilution. UV absorbance of standard solutions was measured by UV spectrophotometer at 445 nm. Riboflavin-5-phosphate standard curve is presented in Figure 6.2.

Drug Content Assay

Drug content assay was performed in triplicate. An exact amount (100-150 mg) of coated pellets was weighed and transferred to 100-ml volumetric flasks. Phosphate buffer was used to dissolve drug pellets. Either a stirring bar or a sonicator bath was used to facilitate the disintegration and dissolution. After drug pellets were completely disintegrated, the samples were centrifuged at 3000 rpm for 10-20 minutes. Supernatant was then collected and measured by UV spectrophotometer at 445 nm. The amount of riboflavin-5-phosphate contained in each formulation was determined using a standard curve.
**Ranitidine Hydrochloride Assay**

**Standard Curves of Ranitidine**

An exact amount (125.0 mg) of ranitidine hydrochloride was weighed and transferred to a 250-ml volumetric flask. The sample was dissolved in simulated gastric fluid (pH 1.4) or phosphate buffer (pH 6.0) and adjusted to the final volume. This stock solution had ranitidine hydrochloride concentration of 500 µg/ml. A series of standard solutions with a concentration of 2.5-60 µg/ml (2.5-150 µg/ml in simulated gastric fluid) was prepared from the stock solution by dilution. UV absorbance of standard solutions was measured by UV spectrophotometer at 330 nm. Ranitidine hydrochloride standard curves are presented in Figures 6.3 and 6.4.

**Drug Content Assay**

Drug content assay was performed in triplicate. An exact amount (150-200 mg) of coated pellets was weighed and transferred to 100-ml volumetric flasks. Phosphate buffer was used to dissolve drug pellets. Either a stirring bar or a sonicator bath was used to facilitate the disintegration and dissolution. After drug pellets were completely disintegrated, the samples were centrifuged at 3000 rpm for 10-20 minutes. Supernatant was then collected and measured by UV spectrophotometer at 330 nm. The amount of ranitidine hydrochloride contained in each formulation was determined using an appropriate standard curve.
Hydrochlorothiazide Assay

Standard Curves of Hydrochlorothiazide

An exact amount (50.0 mg) of hydrochlorothiazide was weighed and transferred to a 250-ml volumetric flask. The sample was dissolved in a mixture (1:5) of ethanol and simulated gastric fluid (pH 1.4) or phosphate buffer (pH 6.0) and adjusted to the final volume. This stock solution had hydrochlorothiazide concentration of 200 µg/ml. A series of standard solutions with a concentration of 1-50 µg/ml was prepared from the stock solution by dilution. UV absorbance of standard solutions was measured by UV spectrophotometer at 318 nm. Hydrochlorothiazide standard curves are presented in Figures 6.5 and 6.6.

Drug Content Assay

Drug content assay was performed in triplicate. An exact amount (approximately 100 mg) of coated pellets was weighed and transferred to 100-ml volumetric flasks. Phosphate buffer was used to dissolve drug pellets. Either a stirring bar or a sonicator bath was used to facilitate the disintegration and dissolution. After drug pellets were completely disintegrated, the samples were centrifuged at 3000 rpm for 10-20 minutes. Supernatant was then collected and measured by UV spectrophotometer at 318 nm. The amount of hydrochlorothiazide contained in each formulation was determined using an appropriate standard curve.
Figure 6.2 Standard curve of riboflavin-5-phosphate in simulated gastric fluid (UV wavelength at 445 nm)

Absorbance = 0.023*Concentration (mcg/ml)

R² = 1.000
Figure 6.3 Standard curve of ranitidine hydrochloride in simulated gastric fluid (UV wavelength at 330 nm)

Absorbance = 0.0024*Concentration (mcg/ml)
R² = 1.000
Figure 6.4 Standard curve of ranitidine hydrochloride in phosphate buffer pH 6.0 (UV wavelength at 330 nm)

Absorbance = 0.0306*Concentration (mcg/ml)

$R^2 = 1.000$
Figure 6.5 Standard curve of hydrochlorothiazide in simulated gastric fluid (UV wavelength at 318 nm)
Figure 6.6 Standard curve of hydrochlorothiazide in phosphate buffer pH 6.0 (UV wavelength at 318 nm)
In Vitro Dissolution Testing of Studied Formulations

In vitro drug release profiles of studied formulations were obtained using the United States Pharmacopeia (USP) XXV dissolution apparatus I, basket method (VK 7000, Vankel Industries, Inc., Cary, NC). Dissolution was studied at a basket rotation speed of 100 rpm and temperature of dissolution bath was maintained at 37.5°C. Dissolution testing of all formulations was performed in triplicate.

Studied formulations were placed into dissolution baskets, which were then immersed in dissolution vessels containing 600 ml of simulated gastric fluid. Dissolution testing was run in simulated gastric fluid for 2 hours. At the end of 2-hour period, the dissolution baskets were transferred into 600 ml of phosphate buffer pH 6.0. Dissolution testing was continued in phosphate buffer until studied formulations were completely disintegrated.

Five (5) ml of samples were manually collected without medium replacement at 0.17, 0.33, 0.5, 0.75, 1, 2, 2.08, 2.17, 2.25, 2.5, 2.75, 3, 4, and 5 hours. The samples were centrifuged at 3000 rpm for 20 minutes. Supernatant was then collected and measured by UV spectrophotometer at 445, 330, and 318 nm for riboflavin, ranitidine, and hydrochlorothiazide, respectively. The amount of drug released was determined using an appropriate standard curve.

Average drug releases and their standard deviations were calculated from three replications in all dissolution experiments. Dissolution profiles are presented as percent drug release versus time curves.
Simulation of Plasma Concentration-Time Profiles from Leaky Enteric-Coated Pellets Formulations

**Pharmacokinetic Models of Leaky Enteric-Coated Pellets**

Compartmental diagrams illustrating pharmacokinetics of drugs from leaky enteric-coated pellets in the fasted and fed condition are created and shown in Figures 6.7 and 6.8, respectively.

![Compartmental diagram of pharmacokinetic model for leaky enteric-coated pellets in fasted condition.](image)

**Figure 6.7** Compartmental diagram of pharmacokinetic model for leaky enteric-coated pellets in fasted condition.

**Notation:** $X_{PS}$, amount of drug in pellets form in the stomach; $X_{SS}$, amount of dissolved drug in the stomach; $X_{SI}$, amount of dissolved drug in the intestine; $X_1$, amount of drug in plasma/blood; **Dose**, a leaky enteric-coated dose; $k_{em}$, a first-order rate of drug input into the intestine corresponding to the first-order gastric emptying of pellets in fasted condition; $k_r$, a first-order release rate of drug from pellets within the stomach; $k_s$, a first-order rate of drug input into the intestine corresponding to the first-order gastric emptying of liquid; $k_a$, a first-order absorption rate constant of drug; $k_{el}$, a first-order elimination rate constant of drug.
Figure 6.8 Compartmental diagram of pharmacokinetic model for leaky enteric-coated pellets in fed condition.

Notation: $X_{PS}$, amount of drug in pellets form in the stomach; $X_{SS}$, amount of dissolved drug in the stomach; $X_{SI}$, amount of dissolved drug in the intestine; $X_1$, amount of drug in plasma/blood; Dose, a leaky enteric-coated dose; $k_0$, a zero-order rate of drug input into the intestine corresponding to the zero-order gastric emptying of pellets in fed condition; $k_r$, a first-order release rate of drug from pellets within the stomach; $k_s$, a first-order rate of drug input into the intestine corresponding to the first-order gastric emptying of liquid; $k_a$, a first-order absorption rate constant of drug; $k_{el}$, a first-order elimination rate constant of drug.

Differential equations describing above compartmental diagram in fasted condition and derivations of pharmacokinetic model of leaky enteric-coated pellets are elaborated in Appendix 6. Pharmacokinetic model of leaky enteric-coated pellets in fasted condition is presented in Equation 6.1.
\[ C_1 = \frac{k_a D}{V} \left[ \frac{(k_c k_s - k_{em} k_c) e^{-k_c t}}{(k_a - k_c)(k_s - k_c)(k_{cl} - k_c)} \right] + \frac{(k_c k_s - k_{em} k_s) e^{-k_s t}}{(k_a - k_s)(k_c - k_s)(k_{cl} - k_c)} + \frac{(k_c k_s - k_{em} k_a) e^{-k_a t}}{(k_s - k_a)(k_c - k_a)(k_{cl} - k_a)} \]

where “\( C_1 \)” is concentration of drug in blood compartment at time \( t \).
“\( V \)” is the volume of distribution.
“\( D \)” is administered dose.
\( k_c = k_{em} + k_r \)

For the fed condition, computer programming codes were developed using MATLAB computer language (The MathWorks, Inc., Natick, MA) to delineate the compartmental diagram. An example of MATLAB programming codes (M-script) describing pharmacokinetics of leaky enteric-coated pellets in fed condition are shown in Appendix 7.

**Model Assumptions**

Assumptions underlying pharmacokinetic models of leaky enteric-coated pellets used in simulations are:

1) Pharmacokinetics of drug are linear in the dosing range of interest. Thus, superposition for determination of plasma drug concentrations can be applied.

2) Leaky enteric-coated formulation is in multi-unit pellet/granule (multi-particulate) form.

3) Drug release from leaky enteric-coated formulation in the stomach is a first-order process.
4) Upon transfer into the intestine, drug release from leaky enteric-coated formulation in the intestine is instantaneous.

5) Once being released from the formulation into the intestine, the drug is absorbed by a first-order process.

6) Pharmacokinetics of the drug in the body are well described by a one-compartment open model.

7) The elimination of the drug from the body is a first-order process.

**Model Parameters**

Pharmacokinetic parameters of riboflavin-5-phosphate, ranitidine hydrochloride, and hydrochlorothiazide used in simulations were obtained by fitting of plasma concentration-time data from the literature (11, 15, 21). All data fittings were performed on data from immediate-release formulations using WinNonlin Professional software, version 3.2 (Pharsight Corporation, Mountain View, CA). Table 6.8 summarizes pharmacokinetic parameters of all model drugs used in simulations.

Bioavailability of 60 mg immediate-release riboflavin was 36.4% (11). Bioavailability of 60 mg riboflavin from leaky enteric-coated pellets used in simulations was assumed to be 85% based on data from gastric retention formulations (2). Bioavailability of leaky enteric-coated pellets of ranitidine hydrochloride was assumed to be equal to that of immediate-release formulation. Bioavailability of 100 mg immediate-release hydrochlorothiazide was 50.3% (15). Bioavailability of 100 mg hydrochlorothiazide from leaky enteric-coated pellets was assumed to be 100% in simulations to demonstrate the maximal theoretical effect.
GI transit parameters involved in simulations were gastric emptying of pellets and gastric emptying of liquid. Gastric emptying of drug pellets in fasted and fed condition are first-order and zero-order processes, respectively (22, 23). Gastric emptying of liquid was a first-order process (24) and was assumed to be at similar rate for both fasted and fed simulations. GI transit parameters used in simulations are shown in Table 6.9.

Table 6.8 Pharmacokinetic Parameters of Model Drugs Used in Simulations

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>F</th>
<th>V (L)</th>
<th>$k_a$ (hr$^{-1}$)</th>
<th>$k_{el}$ (hr$^{-1}$)</th>
<th>$k_r$ (hr$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>85%</td>
<td>190.7</td>
<td>3.67</td>
<td>0.32</td>
<td>0.144, 0.347, 0.693</td>
</tr>
<tr>
<td><strong>Riboflavin-5-phosphate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>IR$^a$</td>
<td>199.6$^b$</td>
<td>0.641</td>
<td>0.239</td>
<td>0.144, 0.347, 0.693</td>
</tr>
<tr>
<td><strong>Ranitidine hydrochloride</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>100%</td>
<td>105.9</td>
<td>0.94</td>
<td>0.13</td>
<td>0.144, 0.347, 0.693</td>
</tr>
<tr>
<td><strong>Hydrochlorothiazide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Bioavailability of ranitidine from leaky formulation was assumed to equal that of IR.
$^b$ This value represents V/F.

Table 6.9 GI Transit Parameters Used in Simulations

<table>
<thead>
<tr>
<th>$k_{em}^a$ (ref.)</th>
<th>Gastric emptying time in fed condition (ref.)</th>
<th>$t_{50}^b$ (ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.386 hr$^{-1}$ (22)</td>
<td>5.7 hr (23)</td>
<td>0.25 hr (24)</td>
</tr>
</tbody>
</table>

$^a$ First-order gastric emptying rate constant of pellets in fasted condition calculated from half-time for gastric emptying (0.5 hr) of pellets in fasted condition
$^b$ Half-time for gastric emptying of liquid
Computer Simulations

All simulations were performed using MATLAB software, version 6.5 (The MathWorks, Inc., Natick, MA). The simulated plasma concentration-time curves of each model drug were visually compared to published literature data of immediate-release formulation.
RESULTS AND DISCUSSION

Dissolution Studies

A dissolution profile of each formulation is presented as an average of three replicates. Vertical bars represent a standard deviation. Vertical bars may not be seen when standard deviation values are very small.

Formulations RF1, RF2, RF3, and RF4 are riboflavin-5-phosphate formulations with enteric-coating layer of Eudragit® L 30 D-55 with no lactose, 50% lactose, 65% lactose, and 83.5% lactose, respectively. Amount of enteric-coating polymer as percent of Eudragit® L 30 D-55 polymer solid on drug-loaded pellets is 5%. Dissolution profiles of RF1 to RF4 are shown in Figure 6.9. Formulation with no lactose (RF1) released riboflavin approximately 5% at 2 hours in simulated gastric fluid. Formulations RF2, RF3, and RF4 released the drug approximately 10%, 35%, and 75%, respectively, at 2 hours in simulated gastric fluid. After being transferred into phosphate buffer medium (pH 6.0), all formulations completely released the drug within 20 minutes (Hour 2.33 from the beginning of the dissolution testing). Formulations with higher amount of lactose, as expected, appeared to completely release the drug slightly faster.

Formulations RTD1 to RTD4 are ranitidine hydrochloride formulations with enteric-coating layer of Eudragit® L 30 D-55 with 33% lactose. Formulations RTD5 to RTD7 are ranitidine hydrochloride formulations with enteric-coating layer of Eudragit® L 30 D-55 with 50% lactose. Amount of enteric-coating polymer as percent of Eudragit® L 30 D-55 polymer solid on drug-loaded pellets for RTD1, RTD2, RTD3, and RTD4 is 7.5%, 10%, 12.5%, and 15%, respectively. Amount of enteric-coating polymer as percent of Eudragit® L 30 D-55 polymer solid on drug-
Dissolution profiles of RTD1 to RTD4 are shown in Figure 6.10. Formulations RTD2, RTD3, and RTD4 released ranitidine approximately 80%, 65%, and 10%, respectively, at 2 hours in simulated gastric fluid. Formulation RTD1 did not have sufficient amount of enteric-coating polymer to sustain release of ranitidine in simulated gastric fluid. Ranitidine was released from RTD1 nearly 95% within 30 minutes of dissolution testing, similar to typical immediate-release formulation. After transfer into phosphate buffer medium (pH 6.0), all formulations completely released the drug within 15-30 minutes (Hour 2.25-2.5 from the beginning of the dissolution testing). Based on formulations RTD1 to RTD4, amount of leaky enteric-coating polymer (Eudragit® L 30 D-55 with 33% lactose) as percent of Eudragit® L 30 D-55 polymer solid on drug-loaded pellets should be more than 7.5%, preferably 10% or more, in order for the formulation to have some sustaining effect on a release of ranitidine in simulated gastric fluid.

Dissolution profiles of formulations RTD5 to RTD7 are displayed in Figure 6.11. Formulations RTD6 and RTD7 released ranitidine approximately 95% and 80%, respectively, at 2 hours in simulated gastric fluid while formulation RTD5 did not have sufficient amount of enteric-coating polymer to sustain release of ranitidine in simulated gastric fluid. Similar to RTD1, RTD5 released ranitidine in simulated gastric fluid approximately 95% within 30 minutes of dissolution testing, resembling typical immediate-release formulations. After transfer into phosphate buffer medium (pH 6.0), all formulations completely released the drug within 15-30 minutes (Hour 2.25-2.5 from the beginning of the dissolution testing). Based on formulations RTD5 to RTD7, when Eudragit® L 30 D-55 with 50% lactose is used as leaky enteric-coating polymer, amount of leaky enteric-coating polymer as percent of Eudragit® L 30 D-55 polymer solid on the drug-loaded pellets studied should be more than 12.5% in order for the formulation to have some sustaining effect on release of ranitidine in simulated gastric fluid.
Formulations HCTZ are hydrochlorothiazide formulations with enteric-coating layer of Eudragit® L 30 D-55 with HPMC E5. Enteric-coating layer of formulation HCTZ1 is Eudragit® L 30 D-55 with 20% HPMC and having amount of enteric-coating polymer as percent of Eudragit® L 30 D-55 polymer solid on drug-loaded pellets of 7.5%. Enteric-coating layer of formulations HCTZ2 to HCTZ4 is Eudragit® L 30 D-55 with 5% HPMC. Amount of enteric-coating polymer as percent of Eudragit® L 30 D-55 polymer solid on drug-loaded pellets for HCTZ2, HCTZ3, and HCTZ4 is 5%, 7.5%, and 10%, respectively. Dissolution profiles of formulations HCTZ1 to HCTZ4 are shown in Figure 6.12. Formulations HCTZ2, HCTZ3, and HCTZ4 released hydrochlorothiazide approximately 95%, 75%, and 55%, respectively, at 2 hours in simulated gastric fluid while formulation HCTZ1 rapidly released 95% of the drug within 20 minutes of dissolution testing in simulated gastric fluid. After transfer into phosphate buffer medium (pH 6.0), all formulations completely released the drug within 15-30 minutes (Hour 2.25-2.5 from the beginning of the dissolution testing). Based on formulations HCTZ2 to HCTZ4, when Eudragit® L 30 D-55 with 5% HPMC is used as leaky enteric-coating polymer, amount of leaky enteric-coating polymer as percent of Eudragit® L 30 D-55 polymer solid on these drug-loaded pellets needs to be more than 5% in order for the formulation to have some sustaining effect on release of hydrochlorothiazide in simulated gastric fluid.

Preparation of leaky enteric-coated pellets using a spray-coating method worked well. The coating process was simple and could be scaled up with ease. Eudragit® L 30 D-55 polymer was selected in formulations because the polymer dissolved in the duodenal pH (pH 5.5 or higher). Dissolution of polymer at this pH allows the formulation to rapidly disintegrate and instantaneously release drugs before it passes an upper intestinal region.
Figure 6.9 In vitro dissolution profiles of riboflavin-5-phosphate from leaky enteric-coated pellet formulations (RF1 to RF4).
Figure 6.10  In vitro dissolution profiles of ranitidine hydrochloride from leaky enteric-coated pellet formulations (RTD1 to RTD4). Leaky enteric-coating polymer in all formulations comprises 33% lactose.
Figure 6.11: In vitro dissolution profiles of ranitidine hydrochloride from leaky enteric-coated pellet formulations (RTD5 to RTD7). Leaky enteric-coating polymer in all formulations comprises 50% lactose.
Figure 6.12 In vitro dissolution profiles of hydrochlorothiazide from leaky enteric-coated pellet formulations (HCTZ1 to HCTZ4). Leaky enteric-coating polymer in HCTZ1 comprises 20% HPMC. Leaky enteric-coating polymer in HCTZ2 to HCTZ4 comprises 5% HPMC.
Computer Simulations

Three different values of a first-order release rate of drug from pellets in the stomach ($k_r$) were used in simulations for all model drugs: 0.144, 0.347, and 0.693 hr$^{-1}$ and they corresponded to an amount of drug release in the stomach at 2 hours of 25%, 50%, and 75%, respectively.

Riboflavin-5-Phosphate

Simulated plasma concentrations of riboflavin-5-phosphate from leaky enteric-coated pellets are presented in Figure 6.13. The simulated plasma concentration profiles were visually compared with observed data of immediate-release riboflavin in fed condition from the literature (11). Bioavailability of 60-mg IR riboflavin in fed condition was 36.4% (11). Bioavailability of riboflavin used in the simulations was 85%. This value was based on a maximum bioavailability of riboflavin achieved from gastric retention formulations of riboflavin previously studied in the authors' laboratory (2). The simulated plasma concentration profiles showed a more prominent sustained-release pattern when the rate of drug release from the leaky formulation in the stomach or the "leakage rate" is slow (i.e. slower than 50% release at 2 hours) or the formulation is administered in the fed condition.

The concept of increasing bioavailability of riboflavin using leaky enteric-coated pellets is to make riboflavin available at the upper intestinal tract slowly enough to not saturate the absorption site. The fast "leakage rate" formulation in the fasted condition seems to be furthest from ideal conditions. Therefore, bioavailability of riboflavin from leaky enteric-coated pellets will probably not be as high as 85% for all formulations. The exact answer for what extent a leaky
enteric-coated pellets formulation can increase bioavailability of riboflavin is still to be determined in currently planned experiments.

**Ranitidine Hydrochloride**

Simulated plasma concentrations of ranitidine hydrochloride from leaky enteric-coated pellets are presented in Figure 6.14. The simulated plasma concentration profiles were visually compared with observed data of immediate-release ranitidine in fasted condition from the FDA database (21). Bioavailability of ranitidine from leaky enteric-coated pellets used in the simulation was equal to bioavailability of an immediate-release formulation of ranitidine. Ranitidine absorption has been previously studied (8). The upper small intestine and jejunum were found to be primary absorption sites of ranitidine while the drug absorption decreased significantly (over 7 times compared to in the jejunum) in the cecum (8). The bioavailability of ranitidine therefore can be decrease when made into sustained-release formulation because some of the drug will still remain in the sustained-release formulation when it arrives in the lower intestinal tract. The assumption for bioavailability of ranitidine from leaky enteric-coated pellets in the simulation was based on the expectation that leaky enteric-coated pellets formulation will help retain the drug in the stomach for a longer time than for an immediate-release dosage form followed by immediate release in the upper intestine and, as a result, prevent the drug from entering a region of poor absorption in the lower intestinal tract.

Unlike riboflavin, the difference in "leakage rate" is not expected to have much different effects on bioavailability of ranitidine. It is expected that the bioavailability of ranitidine from any leaky enteric-coated pellets presented here will be similar to that from immediate-release formulation. The effect on
pharmacokinetics of ranitidine caused by leaky enteric-coated pellets formulation is on the absorption process. Since the drug will slowly be available at the absorption sites due to slow emptying of drug pellets, the absorption process will be sustained as shown in Figure 6.14.

**Hydrochlorothiazide**

Simulated plasma concentrations of hydrochlorothiazide from leaky entericoxidated pellets are presented in Figure 6.15. The simulated plasma concentration profiles were visually compared with observed data of immediate-release hydrochlorothiazide in fasted condition from the literature (15). Bioavailability of 100-mg IR hydrochlorothiazide in fasted condition was 50.3% (15). Bioavailability of hydrochlorothiazide used in the simulations was 100%. This value was based on a desire to see the maximum effect possible but may not necessarily be attained in vivo.

Similar to the riboflavin case, the concept of increasing bioavailability of hydrochlorothiazide using leaky enteric-coated pellets is to make the drug available at an upper intestinal tract slowly enough to not saturate the absorption site, but also to not saturate solubility in the best of cases. The fast “leakage rate” formulation in the fasted condition should have the smallest improvement on the bioavailability. Thus, bioavailability of hydrochlorothiazide from leaky entericoxidated pellets will probably not be as high as 100%, especially not in all formulations.
Figure 6.13 Simulated plasma concentrations of riboflavin-5-phosphate from leaky enteric-coated pellets. Observed data (●) of immediate-release riboflavin in fed condition were from the literature (11).
Figure 6.14  Simulated plasma concentrations of ranitidine hydrochloride from leaky enteric-coated pellets. Observed data (●) of immediate-release ranitidine in fasted condition were from the abbreviated new drug application database at the FDA website (21).
Figure 6.15  Simulated plasma concentrations of hydrochlorothiazide from leaky enteric-coated pellets. Observed data (●) of immediate-release hydrochlorothiazide in fasted condition were from the literature (15).
CONCLUSIONS

Leaky enteric-coated pellets formulations of riboflavin-5-phosphate, ranitidine hydrochloride, and hydrochlorothiazide were successfully prepared by spray-coating. Leaky enteric coats were formulated using a commonly used enteric polymer, Eudragit® L 30 D-55, combined with soluble compound/polymer, lactose or HPMC. The rate of drug release from the formulations in simulated gastric fluid was controlled by modifying the leaky enteric coat content and/or varying coating amount. All leaky enteric-coated formulations studied completely released the model drugs within 30 minutes after changing dissolution medium to phosphate buffer pH 6.0.

Computer simulations were performed in order to predict plasma concentration-time profiles of model drugs from leaky enteric-coated pellets with various “leakage rates” in both fed and fasted conditions. It was hypothesized that leaky enteric-coated pellets formulations were able to provide sustained-release effect on plasma concentration profiles of drugs such as ranitidine without jeopardizing their bioavailability while, in some cases, provided sustained-release effect with improved bioavailability of other drugs such as riboflavin and hydrochlorothiazide.

The present research demonstrates a new use of knowledge about GI transit in drug formulations. It also showed that enteric polymers have uses in areas other than traditional enteric-coated formulations. In this research, the purpose of enteric-coating polymer was not to protect the drugs from stomach acid. Instead, it was used as a controlled-release polymer that worked only in the stomach but not in the intestine. The hypothesis that a leaky enteric-coated pellets formulation can
increase bioavailability of drugs that have a window of absorption is being studied further in vivo.
REFERENCES


CHAPTER 7

GENERAL CONCLUSION

Effects of gastrointestinal transit on plasma concentrations of drugs from enteric-coated pellet formulations were demonstrated. Pharmacokinetic models describing plasma concentrations of drugs from various enteric-coated pellet formulations were proposed. Gastric emptying time, lag time of emptying, and drug release rate from pellets in the small intestine, along with other pharmacokinetic parameters of drugs, were used to construct pharmacokinetic models. The models were evaluated by comparing simulated plasma concentrations of model drugs from Monte Carlo simulations to observed plasma concentrations from the literature. Results showed that the model described plasma concentrations of drugs from enteric-coated pellet formulations very well.

Pharmacokinetic models describing plasma concentrations of drug from mixed immediate-release and enteric-coated pellet formulations were also used in simulations of bioequivalence study results. Effects of drug release rate from enteric-coated pellets in the intestine and its' variability on probability of passing bioequivalence studies were considered. Results from the research are very useful in designing products of enteric pellet formulation, and in refining or selecting the final product for actual bioavailability/bioequivalence study.

Formulations of crushable enteric-coated dosage form were presented. Crushable enteric-coated formulations of amphetamine multi-layered spray-coated enteric-coated drug pellets were prepared, and then compressed into crushable tablets. Dissolution testing of both non-crushed tablets and crushed tablets showed that the intact crushable tablet formulations and the crushed tablet formulations
were both able to prevent the majority of drug from being released into simulated gastric dissolution medium in 2 hours. Even though none of the studied formulations were able to fulfill USP XXIII requirements regarding maximum amount of drug release allowed in acidic medium at 2 hours, which is limited to 10% or less, results from the present research are very promising for future development of crushable/chewable enteric-coated formulations.

Concept and formulations of “leaky” enteric-coated pellets were presented. “Leaky enteric-coated pellet formulation” is enteric-coated pellets that allow some of the drug to be released from the formulation in acidic dissolution medium. Different approaches of making leaky enteric-coated pellets using spray-coating techniques were presented. Plasma concentrations of drug from leaky enteric-coated pellet formulations both in fed and fasted subjects were simulated using computer simulations. Effects of varying leakage rate of pellets in the stomach on predicted plasma concentration profiles were shown. The present research was based on the hypotheses that leaky enteric-coated pellets formulations may be able to provide a “sustained-release” type of effect on plasma concentration profiles of drugs including those with an absorption window (such as ranitidine) without jeopardizing their bioavailability. In some cases a sustained-release type of effect may occur with improved bioavailability of other drugs such as riboflavin and hydrochlorothiazide.
Abbreviated New Drug Application (ANDA) 074-467 Ranitidine hydrochloride

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Appendix 1

Derivation of Pharmacokinetic Models for Enteric-Coated Pellets in Fed Condition
(One-Compartment Models)

![Compartmental diagram](image)

**Figure A.1** Compartmental diagram of one-compartment pharmacokinetic model for enteric-coated pellets in fed condition. $X_{PS}$, amount of drug in the form of pellets in the stomach; $X_{Pl}$, amount of drug in the form of pellets in the intestine; $X_{SI}$, amount of released drug in the intestine; $X_1$, amount of drug in plasma/blood; $k_0$, a zero-order input of drug into the intestine corresponding to zero-order gastric emptying of enteric-coated pellets in fed condition; $k_r$, a first-order release rate of drug from pellets; $k_a$, a first-order absorption rate constant of drug; $k_{el}$, a first-order elimination rate constant of drug.

Three differential equations describing compartmental scheme above are

\[
\frac{dX_{Pl}}{dt} = k_0 - k_r X_{Pl} \quad \text{A.1.1}
\]

\[
\frac{dX_{SI}}{dt} = k_r X_{Pl} - k_a X_{SI} \quad \text{A.1.2}
\]

\[
\frac{dX_1}{dt} = k_a X_{SI} - k_{el} X_1 \quad \text{A.1.3}
\]

Laplace transform of Equation A.1.1 and rearrange

\[
\bar{X}_{Pl} = \frac{k_0}{s(s + k_r)} \quad \text{A.1.4}
\]
Take antiLaplace

\[ X_{p1} = \frac{k_0}{k_r} \left(1 - e^{-k_r t}\right) \]  

A.1.5

Substitute Equation A.1.5 into Equation A.1.2

\[ \frac{dX_{s1}}{dt} = k_0 - k_0 e^{-k_r t} - k_a X_{s1} \]  

A.1.6

Laplace transform Equation A.1.6 and rearrange

\[ \tilde{X}_{s1} = \frac{k_0 k_r}{s(k_r - k_a)} \]  

A.1.7

Take antiLaplace

\[ X_{s1} = k_0 k_r \left[ \frac{1}{k_r k_a} + \frac{e^{-k_r t}}{k_r (k_r - k_a)} - \frac{e^{-k_a t}}{k_a (k_r - k_a)} \right] \]  

A.1.8

Substitute Equation A.1.8 into Equation A.1.3

\[ \frac{dX_1}{dt} = k_s k_0 k_r \left[ \frac{1}{k_r k_a} + \frac{e^{-k_r t}}{k_r (k_r - k_a)} - \frac{e^{-k_a t}}{k_a (k_r - k_a)} \right] - k_{el} X_1 \]  

A.1.9

\[ \frac{dX_1}{dt} = k_0 + \frac{k_0 k_a}{(k_r - k_a)} e^{-k_r t} - \frac{k_0 k_r}{(k_r - k_a)} e^{-k_a t} - k_{el} X_1 \]  

A.1.10

Laplace transform Equation A.1.10

\[ s \tilde{X}_1 - 0 = \frac{k_0}{s} + \frac{k_0 k_a}{(k_r - k_a)(s + k_r)} - \frac{k_0 k_r}{(k_r - k_a)(s + k_a)} - k_{el} \tilde{X}_1 \]  

A.1.11

Rearrange and solve for \( \tilde{X}_1 \)

\[ \tilde{X}_1 = \frac{k_0 k_r k_a}{s(s + k_r)(s + k_a)(s + k_{el})} \]  

A.1.12

Take antiLaplace
Equation A.1.15 is valid as long as zero-order input does not cease. That is it can be used to calculate concentration of the drug in $X_1$ compartment when $t \leq \tau$ gastric emptying time ($\tau$) in fed condition. When gastric emptying of drug pellets has stopped, there are three processes to be considered accordingly.

**Part 1:** The remaining drug in pellets ($X_{P1}$ compartment) at time $\tau$ will be released into the small intestine ($X_{SI}$ compartment) with rate of $k_r$ and then will be absorbed into the blood circulation ($X_1$ compartment) with rate of $k_a$ before being excreted from the body with rate of $k_{el}$.

**Part 2:** The remaining drug solution in the small intestine ($X_{SI}$ compartment) at time $\tau$ will be absorbed into the blood circulation ($X_1$ compartment) with rate of $k_a$ and excreted from the body with rate of $k_{el}$.

**Part 3:** The remaining drug in the blood circulation at time $\tau$ will be excreted with rate of $k_{el}$.
Figure A.2  Compartmental diagram of one-compartment pharmacokinetic models for enteric-coated pellets in fed condition after gastric emptying stopped (Part 1).

Amount of the drug remaining as pellets ($X_{pl}$ compartment) is calculated by using Equation A.1.15. When $t = \tau$, the amount of drug in $X_{pl}$ compartment is determined from

$$X_{pl}(\text{when } t = \tau) = \frac{k_0}{k_r}(1 - e^{-k_r \tau})$$

Plasma concentration of the drug in $X_1$ compartment now can be calculated using an equation of a one-compartment open model with two consecutive first order absorptions (Wagner, J.G.) by substituting a dose with Equation A.1.16. Time parameter in the equation will be time period after discontinuation of the gastric emptying (i.e. $t-\tau$).

$$C_1(\text{Part 1}) = \frac{k_r k_a}{V} \left[ \frac{k_0}{k_r} \left(1 - e^{-k_r \tau}\right) \right] \left[ \frac{e^{-k_r (t-\tau)}}{(k_a - k_r)(k_{cl} - k_r)} + \frac{e^{-k_a (t-\tau)}}{(k_r - k_a)(k_{cl} - k_a)} \right] + \frac{e^{-k_{el} (t-\tau)}}{(k_r - k_{el})(k_a - k_{el})}$$

A.1.17
\[
C_1 (\text{Part 1}) = \frac{k_0k_a}{V} \left(1 - e^{-k_r \tau}\right) \left[\frac{e^{-k_a(t-\tau)}}{(k_a - k_r)(k_{el} - k_r)} + \frac{e^{-k_a(t-\tau)}}{(k_r - k_a)(k_{el} - k_a)}\right] + \frac{e^{-k_a(t-\tau)}}{(k_r - k_a)(k_{el} - k_a)} + \frac{e^{-k_a(t-\tau)}}{(k_r - k_{el})(k_a - k_{el})}\]
\]

A.1.18

**Part 2**

Amount of the drug solution, which is remaining in \(X_{SI}\) compartment, will serve as a starting dose of a one-compartment open model with first order absorption below.

![Diagram](image)

**Figure A.3** Compartmental diagram of one-compartment pharmacokinetic models for enteric-coated pellets in fed condition after gastric emptying stopped (Part 2).

Amount of the drug in \(X_{SI}\) compartment at \(t = \tau\) is calculated using Equation A.1.18 as followed.

\[
X_{SI} \left(\text{when } t = \tau\right) = k_0k_a \left[\frac{1}{k_r k_a} + \frac{e^{-k_r \tau}}{k_r (k_r - k_a)} - \frac{e^{-k_r \tau}}{k_a (k_r - k_a)}\right]
\]

A.1.19
By substituting Equation A.1.19 into an equation for a one-compartment open model with first order absorption, plasma concentration of the drug in $X_1$ compartment can be calculated from

$$C_1\text{(Part 2)} = \frac{k_a k_d k_r}{V (k_a - k_{el})} \left[ \frac{1}{k_r k_a} + \frac{e^{-k_r \tau}}{k_r (k_r - k_a)} \right]$$

$$\quad - \frac{e^{-k_a \tau}}{k_a (k_r - k_a)} \left[ e^{-k_{el} (t - \tau)} - e^{-k_a (t - \tau)} \right]$$

**Part 3**

The amount of the drug previously existing in $X_1$ compartment will decrease with rate constant of $k_{el}$ as following.

$$C_1\text{(Part 3)} = (C_1 \text{ at } t = \tau) e^{-k_{el} (t - \tau)}$$

**Figure A.4** Compartmental diagram of one-compartment pharmacokinetic models for enteric-coated pellets in fed condition after gastric emptying stopped (Part 3).
Therefore, plasma concentration of the drug from enteric-coated pellets that possess first-order release pattern in the intestine can be calculated using following equations.

When \( t \leq \tau \), use Equation A.1.15.

When \( t > \tau \), use Equation A.1.18 + A.1.20 + A.1.22.

When lag time of gastric emptying is accounted for, Equations A.1.15, A.1.18, A.1.20, and A.1.22 are modified to Equations A.1.23-A.1.26, respectively.

\[
C_1(\text{lag} < t \leq \tau + \text{lag}) = \frac{k_0}{V \cdot k_{el}} \left[ 1 - \frac{k_a k_{el} e^{-k_r(t-\text{lag})}}{(k_a - k_r)(k_{el} - k_r)} \right. \\
\left. - \frac{k_r k_{el} e^{-k_r(t-\text{lag})}}{(k_r - k_a)(k_{el} - k_a)} \right. \\
\left. - \frac{k_r k_a e^{-k_{el}(t-\text{lag})}}{(k_r - k_{el})(k_a - k_{el})} \right] e^{-k_{el}(t-\tau)}
\]

\[
C_1(\text{Part 1}) = \frac{k_0 k_a}{V} \left[ (1 - e^{-k_r \tau}) \right. \\
\left. + \frac{e^{-k_r(\tau-\text{lag})}}{(k_a - k_r)(k_{el} - k_r)} \right. \\
\left. + \frac{e^{-k_{el}(\tau-\text{lag})}}{(k_r - k_{el})(k_a - k_{el})} \right]
\]
Hence, plasma concentration of the drug in the presence of gastric emptying lag time can be calculated using following equations.

When \( t \leq \text{lag} \), \( C_1 = 0 \).

When \( \text{lag} < t \leq \tau + \text{lag} \), use Equation A.1.23.

When \( t > \tau + \text{lag} \), use Equations A.1.24 + A.1.25 + A.1.26.
Appendix 2

Derivation of Pharmacokinetic Models for Enteric-Coated Pellets in Fasted Condition (One-Compartment Models)

Figure A.5 Compartmental diagram of one-compartment pharmacokinetic model for enteric-coated pellets in fasted condition. $X_{PS}$, amount of drug in the form of pellets in the stomach; $X_{Pl}$, amount of drug in the form of pellets in the intestine; $X_{Sl}$, amount of released drug in the intestine; $X_1$, amount of drug in plasma/blood; $k_{em}$, a first-order rate of drug input into the intestine corresponding to first-order gastric emptying of enteric-coated pellets in fasted condition; $k_r$, a first-order release rate of drug from pellets; $k_a$, a first-order absorption rate constant of drug; $k_{el}$, a first-order elimination rate constant of drug.

Four differential equations describing compartmental scheme above are

$$\frac{dX_{PS}}{dt} = -k_{em}X_{PS} \quad \text{A.2.1}$$

$$\frac{dX_{Pl}}{dt} = k_{em}X_{PS} - k_rX_{Pl} \quad \text{A.2.2}$$

$$\frac{dX_{Sl}}{dt} = k_rX_{Pl} - k_aX_{Sl} \quad \text{A.2.3}$$

$$\frac{dX_1}{dt} = k_aX_{Sl} - k_{el}X_1 \quad \text{A.2.4}$$

Laplace transform of Equation 3.17 and rearrange
Take antiLaplace

\[ X_{PS} = D \cdot e^{-k_{em} t} \]  \hspace{1cm} A.2.6

Substitute Equation A.2.6 into Equation 3.18

\[ \frac{dX_{Pt}}{dt} = k_{em} D \cdot e^{-k_{em} t} - k_r X_{Pt} \]  \hspace{1cm} A.2.7

Laplace transform of Equation A.2.7 and rearrange

\[ \bar{X}_{Pt} = \frac{k_{em} D}{(s + k_{em})(s + k_r)} \]  \hspace{1cm} A.2.8

Take antiLaplace

\[ X_{Pt} = \frac{k_{em} D}{(k_r - k_{em})} \left( e^{-k_{em} t} - e^{-k_k t} \right) \]  \hspace{1cm} A.2.9

Substitute Equation A.2.9 into Equation 3.19

\[ \frac{dX_{Si}}{dt} = \frac{k_r k_{em} D}{(k_r - k_{em})} \left( e^{-k_{em} t} - e^{-k_k t} \right) - k_a X_{Si} \]  \hspace{1cm} A.2.10

Laplace transform of Equation A.2.10 and rearrange

\[ \bar{X}_{Si} = \frac{k_r k_{em} D}{(s + k_{em})(s + k_r)(s + k_a)} \]  \hspace{1cm} A.2.11

Take antiLaplace

\[ X_{Si} = k_r k_{em} D \left[ \frac{e^{-k_{em} t}}{(k_a - k_{em})(k_r - k_{em})} + \frac{e^{-k_k t}}{(k_a - k_r)(k_{em} - k_r)} \right. \]  

\[ \left. + \frac{e^{-k_{em} t}}{(k_r - k_a)(k_{em} - k_a)} \right] \]  \hspace{1cm} A.2.12
Substitute Equation A.2.12 into Equation 3.20

\[
\frac{dX_1}{dt} = k_a k_{em} D \left[ \frac{e^{-k_{em}t}}{(k_a - k_{em})(k_r - k_{em})(k_{el} - k_{em})} + \frac{e^{-k_{rt}t}}{(k_a - k_r)(k_{el} - k_r)} + \frac{e^{-k_{kt}t}}{(k_r - k_a)(k_{el} - k_a)} - k_{el}X_1 \right]
\]

Laplace transform of Equation A.2.13 and rearrange

\[
\tilde{X}_1 = \frac{k_r k_{em} k_a D}{(s + k_{em})(s + k_r)(s + k_a)(s + k_{el})}
\]

Take antiLaplace

\[
X_1 = k_r k_{em} k_a D \left[ \frac{e^{-k_{em}t}}{(k_a - k_{em})(k_r - k_{em})(k_{el} - k_{em})} + \frac{e^{-k_{rt}t}}{(k_a - k_r)(k_{el} - k_r)} + \frac{e^{-k_{kt}t}}{(k_r - k_a)(k_{el} - k_a)} \right]
\]

\[
C_1 = \frac{k_r k_{em} k_a D}{V} \left[ \frac{e^{-k_{em}t}}{(k_a - k_{em})(k_r - k_{em})(k_{el} - k_{em})} + \frac{e^{-k_{rt}t}}{(k_a - k_r)(k_{el} - k_r)} + \frac{e^{-k_{kt}t}}{(k_r - k_a)(k_{el} - k_a)} \right]
\]

In the presence of gastric emptying lag time, Equation A.2.16 can be modified to
\[ C_1 = \frac{k_r k_{em} k_a D}{V} \left[ e^{-k_{em} (t - \text{lag})} \right. \\
+ \frac{e^{-k_r (t - \text{lag})}}{(k_a - k_r)(k_{em} - k_r)(k_{el} - k_r) + e^{-k_{a} (t - \text{lag})}} \right. \\
+ \left. \frac{e^{-k_{a} (t - \text{lag})}}{(k_a - k_{el})(k_r - k_{el})(k_{em} - k_{el})} \right] \\
\]
Appendix 3

Derivation of Pharmacokinetic Models for Enteric-Coated Pellets in Fed Condition
(Two-Compartment Models)

**Figure A.6** Compartmental diagram of two-compartment pharmacokinetic models for enteric-coated pellets in fed condition. $X_{PS}$, amount of drug in the form of pellets in the stomach; $X_{PI}$, amount drug in the form of pellets in the intestine; $X_{SI}$, amount of drug released in the intestine; $X_1$, amount of drug in plasma/blood; **Dose**, an enteric-coated dose; $k_0$, a zero-order input of drug into the intestine corresponding to the zero-order gastric emptying of enteric-coated pellets in fed condition; $k_r$, a first-order release rate of drug from pellets; $k_a$, a first-order absorption rate constant of drug; $k_{12}$ and $k_{21}$, inter-compartmental rate constants; $k_{el}$, a first-order elimination rate constant of drug.

Four differential equations describing compartmental scheme above are

$$\frac{dX_{PI}}{dt} = k_0 - k_r X_{PI} \quad A.2.18$$

$$\frac{dX_{SI}}{dt} = k_r X_{PI} - k_a X_{SI} \quad A.2.19$$
\[
\frac{dX_1}{dt} = k_a X_{sl} + k_{21} X_2 - k_{12} X_1 - k_{el} X_1 \\
\frac{dX_2}{dt} = k_{12} X_1 - k_{21} X_2
\]

Laplace transform of Equation 3.13 and rearrange

\[
\bar{X}_{pl} = \frac{k_0}{s(s + k_r)}
\]

Take antiLaplace

\[
X_{pl} = \frac{k_0}{k_r} \left( 1 - e^{-k_r t} \right)
\]

Substitute Equation A.2.23 into Equation 3.14

\[
\frac{dX_{sl}}{dt} = k_0 - k_0 e^{-k_r t} - k_a X_{sl}
\]

Laplace transform Equation A.2.24 and rearrange

\[
\bar{X}_{sl} = \frac{k_0 k_r}{s(s + k_r)(s + k_a)}
\]

Take antiLaplace

\[
X_{sl} = k_0 k_r \left[ \frac{1}{k_r k_a} + \frac{e^{-k_r t}}{k_r (k_r - k_a)} + \frac{e^{-k_a t}}{k_a (k_a - k_r)} \right]
\]

Substitute Equation A.2.26 into Equation 3.15

\[
\frac{dX_1}{dt} = k_a k_0 k_r \left[ \frac{1}{k_r k_a} + \frac{e^{-k_r t}}{k_r (k_r - k_a)} + \frac{e^{-k_a t}}{k_a (k_a - k_r)} \right]
+ k_{21} X_2 - k_{12} X_1 - k_{el} X_1
\]

\[
\frac{dX_1}{dt} = k_0 + \frac{k_0 k_a}{(k_r - k_a)} e^{-k_r t} + \frac{k_0 k_r}{(k_a - k_r)} e^{-k_a t} + k_{21} X_2 - k_{12} X_1 - k_{el} X_1
\]
Laplace transform Equation A.2.28

\[
s\bar{X}_1 - 0 = \frac{k_0}{s} + \frac{k_0 k_a}{(k_r - k_a)(s + k_r)} + \frac{k_0 k_r}{(k_a - k_r)(s + k_a)} + k_{21}\bar{X}_2 - k_{12}\bar{X}_1 - k_{el}\bar{X}_1
\]

A.2.29

Rearrange

\[
(s + k_{12} + k_{el})\bar{X}_1 - k_{21}\bar{X}_2 = \frac{k_0}{s} + \frac{k_0 k_a}{(k_r - k_a)(s + k_r)} + \frac{k_0 k_r}{(k_a - k_r)(s + k_a)}
\]

A.2.30

Laplace transform of Equation 3.16 and rearrange

\[-k_{12}\bar{X}_1 + (s + k_{21})\bar{X}_2 = 0
\]

A.2.31

From Equation A.2.30 and A.2.31, solve for \(\bar{X}_1\) and \(\bar{X}_2\) using a determinant (\(\Delta\)) and Cramer’s rule.

\[
\Delta = \begin{vmatrix}
(s + k_{12} + k_{el}) & -k_{21} \\
-k_{12} & (s + k_{21})
\end{vmatrix}
\]

A.2.32

\[
\Delta = (s + k_{12} + k_{el})(s + k_{21}) - k_{12}k_{21}
\]

A.2.33

\[
\Delta = s^2 + (k_{12} + k_{21} + k_{el})s + k_{21}k_{el}
\]

A.2.34

Let two roots of above quadratic equation are \(-\alpha\) and \(-\beta\). When \(\Delta = 0\),

\[
\alpha = \frac{1}{2} \left( (k_{12} + k_{21} + k_{el}) + \sqrt{(k_{12} + k_{21} + k_{el})^2 - 4k_{21}k_{el}} \right)
\]

A.2.35

\[
\beta = \frac{1}{2} \left( (k_{12} + k_{21} + k_{el}) - \sqrt{(k_{12} + k_{21} + k_{el})^2 - 4k_{21}k_{el}} \right)
\]

A.2.36

Therefore,

\[
\Delta = s^2 + (\alpha + \beta)s + \alpha\beta = (s + \alpha)(s + \beta)
\]

A.2.37

\[
\alpha\beta = k_{21}k_{el}
\]

A.2.38
Solve for $\bar{X}_1$

\[
\bar{X}_1 = \begin{bmatrix}
\frac{k_0}{s} + \frac{k_0k_a}{(k_r-k_a)(s+k_r)} + \frac{k_0k_r}{(k_a-k_r)(s+k_a)} & -k_{21} \\
0 & (s+k_{21})
\end{bmatrix}
\]

A.2.39

\[
\bar{X}_1 = \frac{k_0k_rk_a(s+k_{21})}{s(s+k_r)(s+k_a)(s+\alpha)(s+\beta)}
\]

A.2.40

Solve for $X_1$ using the general partial fraction theorem

\[
X_1 = \frac{k_0k_{21}}{\alpha \beta} \frac{k_0k_a (k_{21} - k_r) e^{-\alpha t}}{(k_a-k_r)(\alpha-k_r)(\beta-k_r)} - \frac{k_0k_r (k_{21} - k_a) e^{-\beta t}}{(k_r-k_a)(\alpha-k_a)(\beta-k_a)}
\]  

A.2.41

\[
C_1 (\text{when } t \leq \tau) = \frac{k_0k_{21}}{V_1 \alpha \beta} \frac{k_0k_a (k_{21} - k_r) e^{-\alpha t}}{V_1 (k_a-k_r)(\alpha-k_r)(\beta-k_r)} - \frac{k_0k_r (k_{21} - k_a) e^{-\beta t}}{V_1 (k_r-k_a)(\alpha-k_a)(\beta-k_a)}
\]

A.2.42

Solve for $\bar{X}_2$

\[
\bar{X}_2 = \begin{bmatrix}
(k_0 + \frac{k_0k_a}{s} + \frac{k_0k_r}{(k_r-k_a)(s+k_r)} + \frac{k_0k_r}{(k_a-k_r)(s+k_a)}) & 0 \\
-k_{12} & \Delta
\end{bmatrix}
\]

A.2.43

\[
\bar{X}_2 = \frac{k_0k_rk_{12}}{s(s+k_r)(s+k_a)(s+\alpha)(s+\beta)}
\]

A.2.44
Solve for $X_2$ using the general partial fraction theorem

$$X_2 = \frac{k_0 k_{12}}{\alpha\beta} \frac{k_0 k_a^2 k_{12} e^{-k_r t}}{(k_a - k_r)(\alpha - k_r)(\beta - k_r)} - \frac{k_0 k_r k_{12} e^{-k_a t}}{(k_r - k_a)(\alpha - k_a)(\beta - k_a)} - \frac{k_0 k_r k_a^2 k_{12} e^{-\alpha t}}{\alpha (k_r - \alpha)(k_a - \alpha)(\beta - \alpha)} - \frac{k_0 k_r k_a^2 k_{12} e^{-\beta t}}{\beta (k_r - \beta)(k_a - \beta)(\alpha - \beta)}$$  \hspace{1cm} A.2.45

Equation A.2.42 is valid as long as zero-order input does not cease. That is it can be used to calculate concentration of the drug in $X_1$ compartment when $t \leq$ gastric emptying time ($\tau$) in fed condition. When gastric emptying of drug pellets has stopped, there are three processes to be considered accordingly.

**Part 1:** The remaining drug in the blood circulation ($X_1$) and peripheral compartment ($X_2$) at time $\tau$ will be excreted from the body.

**Part 2:** The remaining drug solution in the small intestine ($X_{SI}$ compartment) at time $\tau$ will be absorbed into the body and then excreted.

**Part 3:** The remaining drug in pellets ($X_{PL}$ compartment) at time $\tau$ will be released into the small intestine ($X_{SI}$ compartment) with rate of $k_r$ and then will be absorbed into the body before being excreted.
Figure A.7  Compartmental diagram of two-compartment pharmacokinetic models for enteric-coated pellets in fed condition after gastric emptying stopped (Part 1).

Two differential equations describing compartmental scheme above are

\[ \frac{dX_1}{dt} = k_{21}X_2 - k_{12}X_1 - k_{ei}X_1 \]  \hspace{1cm} A.2.46

\[ \frac{dX_2}{dt} = k_{12}X_1 - k_{21}X_2 \]  \hspace{1cm} A.2.47

Let \( D_1 \) and \( D_2 \) denote amount of drug in \( X_1 \) and \( X_2 \) compartment at \( t = \tau \), respectively. Determine \( D_1 \) and \( D_2 \) using Equations A.2.41 and A.2.45 as following.

\[ D_1 = \frac{k_0k_{21}}{\alpha \beta} - \frac{k_0k_a}{(k_a - k_r)(\alpha - k_r)(\beta - k_r)} e^{-\alpha \tau} - \frac{k_0}{(k_r - k_a)(\alpha - k_a)(\beta - k_a)} e^{-k_{a} \tau} \]

\[ \frac{k_0k_rk_a}{\alpha(k_r - \alpha)(k_a - \alpha)(\beta - \alpha)} - \frac{k_0k_rk_a}{\beta(k_r - \beta)(k_a - \beta)(\alpha - \beta)} e^{-\beta \tau} \]

A.2.48
\[
D_2 = \frac{k_0k_{12}}{\alpha\beta} \frac{k_0k_2e^{-k_2\tau}}{(k_a - k_r)(\alpha - k_r)(\beta - k_r)} \frac{k_0k_2e^{-k_2\tau}}{(k_a - k_r)(\beta - k_a)(\alpha - k_a)}
\]

A.2.49

Laplace transform Equations A.2.46 and A.2.47

\[
s\bar{X}_1 - D_1 = k_{21}\bar{X}_2 - k_{12}\bar{X}_1 - k_{el}\bar{X}_1
\]

A.2.50

\[
s\bar{X}_2 - D_2 = k_{12}\bar{X}_1 - k_{21}\bar{X}_2
\]

A.2.51

Rearrange

\[
(s + k_{12} + k_{el})\bar{X}_1 - k_{21}\bar{X}_2 = D_1
\]

A.2.52

\[-k_{12}\bar{X}_1 + (s + k_{21})\bar{X}_2 = D_2
\]

A.2.53

From Equation A.2.52 and A.2.53, solve for \( \bar{X}_1 \) using a determinant (\( \Delta \)) and Cramer’s rule.

\[
\Delta = \begin{vmatrix}
(s + k_{12} + k_{el}) & -k_{21} \\
-k_{12} & (s + k_{21})
\end{vmatrix}
\]

A.2.54

\[
\Delta = (s + k_{12} + k_{el})(s + k_{21}) - k_{12}k_{21}
\]

A.2.55

\[
\Delta = s^2 + (k_{12} + k_{21} + k_{el})s + k_{21}k_{el}
\]

A.2.56

Let two roots of above quadratic equation are \(-\alpha\) and \(-\beta\). When \(\Delta = 0\),

\[
\alpha = \frac{1}{2}\left( k_{12} + k_{21} + k_{el} + \sqrt{(k_{12} + k_{21} + k_{el})^2 - 4k_{21}k_{el}} \right)
\]

A.2.57

\[
\beta = \frac{1}{2}\left( k_{12} + k_{21} + k_{el} - \sqrt{(k_{12} + k_{21} + k_{el})^2 - 4k_{21}k_{el}} \right)
\]

A.2.58

Therefore,

\[
\Delta = s^2 + (\alpha + \beta)s + \alpha\beta = (s + \alpha)(s + \beta)
\]

A.2.59
\[ \alpha \beta = k_{21} k_e \] \hspace{1cm} A.2.60

Solve for \( \tilde{X}_1 \)

\[
\tilde{X}_1 = \frac{ \begin{vmatrix} D_1 & -k_{21} \\ D_2 & (s + k_{21}) \end{vmatrix} }{\Delta}
\]

\[ \tilde{X}_1 = \frac{D_1 s + k_{21} (D_1 + D_2)}{(s + \alpha)(s + \beta)} \] \hspace{1cm} A.2.61

Take antiLaplace

\[
X_1 = \frac{\left[ k_{21} (D_1 + D_2) - D_1 \alpha \right]}{\beta - \alpha} e^{-\alpha t} + \frac{\left[ k_{21} (D_1 + D_2) - D_1 \beta \right]}{\alpha - \beta} e^{-\beta t}
\] \hspace{1cm} A.2.63

\[
C_1 = \frac{\left[ k_{21} (D_1 + D_2) - D_1 \alpha \right]}{V_1 (\beta - \alpha)} e^{-\alpha t} + \frac{\left[ k_{21} (D_1 + D_2) - D_1 \beta \right]}{V_1 (\alpha - \beta)} e^{-\beta t}
\] \hspace{1cm} A.2.64
Let $D_3$ denote amount of drug in $X_{SI}$ compartment at $t = \tau$. $D_3$ can be determined using Equation A.2.26.

$$D_3 = \frac{k_0}{k_a} + \frac{k_0 e^{-k_r \tau}}{(k_r - k_a)} + \frac{k_0 k_1 e^{-k_{r1} \tau}}{k_a (k_a - k_r)} \tag{A.2.65}$$

Amount of drug in $X_1$ compartment can be calculated using a two-compartment first-order absorption model.

$$X_1 = \frac{k_a D_3 (k_{21} - \alpha) e^{-\alpha t}}{(k_a - \alpha)(\beta - \alpha)} + \frac{k_a D_3 (k_{21} - \beta) e^{-\beta t}}{(k_a - \beta)(\alpha - \beta)} + \frac{k_a D_3 (k_{21} - k_a) e^{-k_{a1} t}}{(\alpha - k_a)(\beta - k_a)} \tag{A.2.66}$$

**Figure A.8** Compartmental diagram of two-compartment pharmacokinetic models for enteric-coated pellets in fed condition after gastric emptying stopped (Part 2).
Let $D_4$ denote amount of drug in $X_{PI}$ compartment at $t = \tau$. $D_4$ can be determined using Equation A.2.23.

$$D_4 = \frac{k_0}{k_r} \left(1 - e^{-k_r \tau}\right)$$

Amount of drug in $X_1$ compartment can be calculated using a two-compartment sequential first-order absorption model (Wagner, J.G.).
Therefore, plasma concentration of the drug from enteric-coated pellets that possess first-order release pattern in the intestine can be calculated using following equations.

When $t \leq \tau$, use Equation A.2.42.

When $t > \tau$, use Equations A.2.64 + A.2.67 + A.2.70.

When lag time of gastric emptying is accounted for, Equations A.2.42, A.2.64, A.2.67, and A.2.70 are modified to Equations 3.22 to 3.25, respectively.
\[ C_1 \text{ (lag} \leq \tau + \text{lag)} = \frac{k_0 k_{21}}{V_1 \alpha \beta} \left( \frac{k_0 k_a (k_{21} - k_r) e^{-k_r (t - \text{lag})}}{V_1 (k_a - k_r) (\alpha - k_a) (\beta - k_r)} \right) - \frac{k_0 k_r (k_{21} - k_a) e^{-k_a (t - \text{lag})}}{V_1 (k_r - k_a) (\alpha - k_a) (\beta - k_a)} \]  

\[ - \frac{k_0 k_r k_a (k_{21} - \alpha) e^{-\alpha (t - \text{lag})}}{V_1 \alpha (k_r - \alpha) (k_a - \alpha) (\beta - \alpha)} \]  

\[ - \frac{k_0 k_r k_a (k_{21} - \beta) e^{-\beta (t - \text{lag})}}{V_1 \beta (k_r - \beta) (k_a - \beta) (\alpha - \beta)} \]  

\[ A.2.71 \]

\[ C_1 \text{ (Part 1)} = \frac{k_{21} (D_1 + D_2) - D_1 \alpha}{V_1 (\beta - \alpha)} e^{-\alpha (t - \text{lag})} \]  

\[ + \frac{k_{21} (D_1 + D_2) - D_2 \beta}{V_1 (\alpha - \beta)} e^{-\beta (t - \text{lag})} \]  

\[ A.2.72 \]

\[ C_1 \text{ (Part 2)} = \frac{k_a D_3 (k_{21} - \alpha) e^{-\alpha (t - \text{lag})}}{V_1 (k_a - \alpha) (\beta - \alpha)} \]  

\[ + \frac{k_a D_3 (k_{21} - \beta) e^{-\beta (t - \text{lag})}}{V_1 (k_a - \beta) (\alpha - \beta)} \]  

\[ + \frac{k_a D_3 (k_{21} - k_a) e^{-k_a (t - \text{lag})}}{V_1 (\alpha - k_a) (\beta - k_a)} \]  

\[ A.2.73 \]
Hence, plasma concentration of the drug in the presence of gastric emptying lag time can be calculated using following equations.

When \( t \leq \text{lag} \), \( C_1 = 0 \).

When \( \text{lag} < t \leq \tau + \text{lag} \), use Equation 3.22.

When \( t > \tau + \text{lag} \), use Equations 3.23 + 3.24 + 3.25.
Appendix 4

Derivation of Pharmacokinetic Models for Enteric-Coated Pellets in Fasted Condition (Two-Compartment Models)

**Figure A.10** Compartment diagram of two-compartment pharmacokinetic models for enteric-coated pellets in fasted condition. \( X_{PS} \), amount of drug in the form of pellets in the stomach; \( X_{PI} \), amount of drug in the form of pellets in the intestine; \( X_{SI} \), amount of drug released in the intestine; \( X_1 \), amount of drug in plasma/blood; Dose, an enteric-coated dose; \( k_{em} \), a first-order rate of drug input into the intestine corresponding to the first-order gastric emptying of enteric-coated pellets in fasted condition; \( k_r \), a first-order release rate of drug from pellets; \( k_a \), a first-order absorption rate constant of drug; \( k_{12} \) and \( k_{21} \), inter-compartmental rate constants; \( k_{el} \), a first-order elimination rate constant of drug.

Five differential equations describing compartmental scheme above are

\[
\frac{dX_{PS}}{dt} = -k_{em}X_{PS} \quad \text{A.4.1}
\]

\[
\frac{dX_{PI}}{dt} = k_{em}X_{PS} - k_rX_{PI} \quad \text{A.4.2}
\]
\[
\frac{dX_S}{dt} = k_r X_p - k_a X_S \\
\frac{dX_1}{dt} = k_a X_S + k_{21} X_2 - k_{12} X_1 - k_d X_1 \\
\frac{dX_2}{dt} = k_{12} X_1 - k_{21} X_2
\]

Laplace transform of equation 3.17 and rearrange

\[
\tilde{X}_{PS} = \frac{D}{s + k_{em}}
\]

Take antiLaplace

\[
X_{PS} = D \cdot e^{-k_{em}t}
\]

Substitute equation A.2.6 into equation 3.18

\[
\frac{dX_p}{dt} = k_{em} D \cdot e^{-k_{em}t} - k_r X_p
\]

Laplace transform of equation A.2.7 and rearrange

\[
\tilde{X}_p = \frac{k_{em} D}{(s + k_{em})(s + k_r)}
\]

Take antiLaplace

\[
X_p = \frac{k_{em} D}{(k_r - k_{em})} \left( e^{-k_{em}t} - e^{-k_r t} \right)
\]

Substitute equation A.2.9 into equation 3.19

\[
\frac{dX_S}{dt} = \frac{k_r k_{em} D}{(k_r - k_{em})} \left( e^{-k_{em}t} - e^{-k_r t} \right) - k_a X_S 
\]

Laplace transform of equation A.2.10 and rearrange
\[ \bar{X}_{SL} = \frac{k_r k_{em} D}{(s + k_{em})(s + k_r)(s + k_a)} \]  \hspace{1cm} \text{(A.4.12)}

Take anti-Laplace

\[ X_{SL} = k_r k_{em} D \left[ \frac{e^{-k_r t} - e^{-k_{em} t}}{(k_a - k_{em})(k_r - k_{em})} + \frac{e^{-k_r t}}{(k_a - k_r)(k_{em} - k_r)} \right] \]  \hspace{1cm} \text{(A.4.13)}

Substitute equation A.2.12 into equation 3.20

\[ \frac{dX_1}{dt} = k_a k_r k_{em} D \left[ \frac{e^{-k_{em} t} - e^{-k_r t}}{(k_a - k_{em})(k_r - k_{em})} + \frac{e^{-k_r t}}{(k_a - k_r)(k_{em} - k_r)} \right] \]  \hspace{1cm} \text{(A.4.14)}

Laplace transform of equation A.2.13 and rearrange

\[ (s + k_{12} + k_{el}) \bar{X}_1 - k_{21} \bar{X}_2 = \frac{k_a k_r k_{em} D}{(s + k_{em})(s + k_r)(s + k_a)} \]  \hspace{1cm} \text{(A.4.15)}

Laplace transform of equation 3.21 and rearrange

\[ -k_{12} \bar{X}_1 + (s + k_{21}) \bar{X}_2 = 0 \]  \hspace{1cm} \text{(A.4.16)}

From equation A.4.15 and A.4.16, solve for \( \bar{X}_1 \) and \( \bar{X}_2 \) using a determinant (\( \Delta \)) and Cramer's rule.

\[ \Delta = \begin{vmatrix} (s + k_{12} + k_{el}) & -k_{21} \\ -k_{12} & (s + k_{21}) \end{vmatrix} \]  \hspace{1cm} \text{(A.4.17)}

\[ \Delta = (s + k_{12} + k_{el})(s + k_{21}) - k_{12} k_{21} \]  \hspace{1cm} \text{(A.4.18)}

\[ \Delta = s^2 + (k_{12} + k_{21} + k_{el}) s + k_{21} k_{el} \]  \hspace{1cm} \text{(A.4.19)}

Let two roots of above quadratic equation are \( -\alpha \) and \( -\beta \). When \( \Delta = 0 \),
\[ \alpha = \frac{1}{2} \left[ (k_{12} + k_{21} + k_{el}) + \sqrt{(k_{12} + k_{21} + k_{el})^2 - 4k_{21}k_{el}} \right] \]  
\[ \beta = \frac{1}{2} \left[ (k_{12} + k_{21} + k_{el}) - \sqrt{(k_{12} + k_{21} + k_{el})^2 - 4k_{21}k_{el}} \right] \]  

Therefore,

\[ \Delta = s^2 + (\alpha + \beta)s + \alpha\beta = (s + \alpha)(s + \beta) \]  

Solve for \( X_1 \)

\[
X_1 = \frac{k_ak_ek_{em}D}{(s + k_{em})(s + k_r)(s + k_a)} \begin{vmatrix} k_{21}k_{em}D & -k_{21} \\ (s + k_{em})(s + k_r)(s + k_a) & 0 \\ \Delta & (s + k_{21}) \end{vmatrix} \]  

\[
X_1 = \frac{k_ak_ek_{em}D(s + k_{21})}{(s + k_{em})(s + k_r)(s + k_a)(s + \alpha)(s + \beta)} \]  

Solve for \( X_1 \) using the general partial fraction theorem

\[
X_1 = k_ak_ek_{em}D \left[ \frac{(k_{21} - k_{em})e^{-k_{em}t}}{(k_a - k_{em})(k_r - k_{em})(\alpha - k_{em})(\beta - k_{em})} + \frac{(k_{21} - k_r)e^{-k_{r}t}}{(k_a - k_r)(k_{em} - k_r)(\alpha - k_r)(\beta - k_r)} + \frac{(k_{21} - k_a)e^{-k_{a}t}}{(k_a - k_a)(k_{em} - k_a)(\alpha - k_a)(\beta - k_a)} + \frac{(k_{21} - \alpha)e^{-\alpha t}}{(k_a - \alpha)(k_r - \alpha)(k_{em} - \alpha)(\beta - \alpha)} + \frac{(k_{21} - \beta)e^{-\beta t}}{(k_a - \beta)(k_r - \beta)(k_{em} - \beta)(\alpha - \beta)} \right] \]
In the presence of gastric emptying lag time, equation A.2.16 can be modified to

\[
C_1 = \frac{k_\alpha k_r k_{em} D}{V_1} \left[ \frac{(k_{21} - k_{em}) e^{-k_{em}t}}{(k_a - k_{em})(k_r - k_{em})(\alpha - k_{em})(\beta - k_{em})} + \frac{(k_{21} - k_r) e^{-k_r t}}{(k_a - k_r)(k_{em} - k_r)(\alpha - k_r)(\beta - k_r)} + \frac{(k_{21} - k_a) e^{-k_a t}}{(k_{em} - k_a)(\alpha - k_a)(\beta - k_a)} + \frac{(k_{21} - \beta) e^{-\beta t}}{(k_a - \beta)(k_r - \beta)(k_{em} - \beta)(\alpha - \beta)} \right]
\]

\[
A.4.26
\]

\[
C_1 = \frac{k_\alpha k_r k_{em} D}{V_1} \left[ \frac{(k_{21} - k_{em}) e^{-k_{em}(t_{lag})}}{(k_a - k_{em})(k_r - k_{em})(\alpha - k_{em})(\beta - k_{em})} + \frac{(k_{21} - k_r) e^{-k_r (t_{lag})}}{(k_a - k_r)(k_{em} - k_r)(\alpha - k_r)(\beta - k_r)} + \frac{(k_{21} - k_a) e^{-k_a (t_{lag})}}{(k_{em} - k_a)(\alpha - k_a)(\beta - k_a)} + \frac{(k_{21} - \beta) e^{-\beta (t_{lag})}}{(k_a - \beta)(k_r - \beta)(k_{em} - \beta)(\alpha - \beta)} \right]
\]

\[
A.4.27
\]
MATLAB Programming Codes for Simulations of Bioequivalence Studies

MATLAB Programming Codes for Simulations of Bioequivalence Studies in Fasted Condition

% This M-Script contains MATLAB commands for performing simulation of
% bioequivalent study in FASTED condition of
% - reference formulation: Adderall XR
% - test formulation: Mixed IR and enteric-coated pellet of amphetamine
% By Prapoch Watanalumlerd (Keng) November 7, 2003

clear all

%ntrials = input('Number of trials? '); % Number of simulated BE trials
disp('start simulation...')
randn('state',sum(100*clock)) % Reset RNG's state

% Define variables
Ts = [0; 1; 2; 3; 4; 5; 6; 7; 8; 9; 10; 11; 12; 14; 16; 24]; % Sampling time
N = 24; % Number of subjects in BE study
MATLAB Programming Codes in Fasted Condition (Continued)

Decision = zeros(ntrials, 1); %Pass/Failure decision storage

AvgLNCmaxDiff = zeros(ntrials, 1); %Difference btw. average LNCmax of test
     %and ref.

CI90_U = zeros(ntrials, 1); %Upper 90% CI bound

CI90_L = zeros(ntrials, 1); %Lower 90% CI bound

EBar = zeros(ntrials, 1); %tinv(0.9, DF)*sqrt(2*MSE/N) storage

%Variables for ref. Cmax analysis

AvgRCmax = zeros(ntrials, 1); %Ref. average Cmax from each trial

StdRCmax = zeros(ntrials, 1); %Ref. Std. of Cmax from each trial

CVRCmax = zeros(ntrials, 1); %Ref. %CV of Cmax from each trial

%Define parameters

IRDose = 4688; %IR dose (in mcg) equivalent to 10000 mcg salts

ECDose = 4688; %EC dose (in mcg) equivalent to 10000 mcg salts

KrR_mu = 3.5; %Mean value for drug release rate constant (EC ref. dose) in pH
     %6.0 buffer

KrR_ERR_CV = 0.05; %Residual CV for KrR

KrT_mu = 0.693; %Mean value for release rate constant (EC test dose) in pH 6.0
     %buffer
MATLAB Programming Codes in Fasted Condition (Continued)

KrT_ERR_CV = 0.05; %Residual CV for KrT

Lag_mu = 0.5; %Population mean value for lag time of emptying of pellets in
%fasted state
Lag_ETA_CV = 0.2; %Lag CV-intersubject
Lag_EPS_CV = 0.15; %Lag CV-intrasubject

Kem_mu = 1.386; %Population mean value for emptying constant of pellets in
%fasted state
Kem_ETA_CV = 0.2; %Kem CV-intersubject
Kem_EPS_CV = 0.15; %Kem CV-intrasubject

Ka_mu = 0.744; %Population mean value for Ka
Ka_ETA_CV = 0.25; %Ka CV-intersubject
Ka_EPS_CV = 0.15; %Ka CV-intrasubject

Kel_mu = 0.067; %Population mean value for Kel
Kel_ETA_CV = 0.25; %Kel CV-intersubject
Kel_EPS_CV = 0.15; %Kel CV-intrasubject

V_mu = 247; %Population mean value for V (in L)
V_ETA_CV = 0.25; %V CV-intersubject
V_EPS_CV = 0.15; %V CV-intrasubject

ERR_CV = 0.05; %Residual (assay, model misspecification) CV
MATLAB Programming Codes in Fasted Condition (Continued)

%Do the simulation ntrials times
for trial = 1:ntrials,
    %Counter
    if rem(ntrials - trial, 200) == 0,
        disp(ntrials - trial)
    end

%Generate PK parameters
KrR = KrR_mu * exp(randn(1,N)*KrR_ERR_CV); %Introduce residual var. (Ref.)
KrT = KrT_mu * exp(randn(1,N)*KrT_ERR_CV); %Introduce residual var. (Test)
Lag = ones(2,1) * Lag_mu * exp(randn(1,N)*Lag_ETA_CV); %Introduce interindividual var.
Lag = Lag .* exp(randn(2,N)*Lag_EPS_CV); %Introduce intraindividual var.
Kem = ones(2,1) * Kem_mu * exp(randn(1,N)*Kem_ETA_CV); %Introduce interindividual var.
Kem = Kem .* exp(randn(2,N)*Kem_EPS_CV); %Introduce intraindividual var.
MATLAB Programming Codes in Fasted Condition (Continued)

\[ Ka = \text{ones}(2,1) \times \text{Ka\_mu} \times \exp(\text{randn}(1,N)\times \text{Ka\_ETA\_CV}); \% \text{Introduce} \]
\[ \text{Ka} = \text{Ka} \times \exp(\text{randn}(2,N)\times \text{Ka\_EPS\_CV}); \% \text{Introduce intraindividual var.} \]

\[ \text{Kel} = \text{ones}(2,1) \times \text{Kel\_mu} \times \exp(\text{randn}(1,N)\times \text{Kel\_ETA\_CV}); \% \text{Introduce} \]
\[ \text{Kel} = \text{Kel} \times \exp(\text{randn}(2,N)\times \text{Kel\_EPS\_CV}); \% \text{Introduce intraindividual var.} \]

\[ \text{V} = \text{ones}(2,1) \times \text{V\_mu} \times \exp(\text{randn}(1,N)\times \text{V\_ETA\_CV}); \% \text{Introduce} \]
\[ \text{V} = \text{V} \times \exp(\text{randn}(2,N)\times \text{V\_EPS\_CV}); \% \text{Introduce intraindividual var.} \]

\% Simulate plasma profile for reference formulation
\[ \text{RKr} = \text{ones}(\text{length(Ts)},1)\times \text{KrR}; \% \text{Prepare parameters for calculation} \]
\[ \text{RKem} = \text{ones}(\text{length(Ts)},1)\times \text{Kem}(1,:); \]
\[ \text{RKa} = \text{ones}(\text{length(Ts)},1)\times \text{Ka}(1,:); \]
\[ \text{RKeI} = \text{ones}(\text{length(Ts)},1)\times \text{Kel}(1,:); \]
\[ \text{RV} = \text{ones}(\text{length(Ts)},1)\times \text{V}(1,:); \]

\[ \text{Tc} = \text{Ts} \times \text{ones}(1,N); \% \text{Generate Ts for each N subject} \]
MATLAB Programming Codes in Fasted Condition (Continued)

%Calculate plasma profile from IR dose
RIRConc = [IRDose*RKa./RV./(RKa-RKel)]. * (exp(-RKel.*Tc)-exp(-RKa.*Tc));

Tc = Tc - ones(length(Ts),1)*Lag(1,:); %Subtract Lag from Ts for each N subject
Tc(Tc < 0) = 0;

%Calculate plasma profile from EC dose
RECConc = (ECDose*RKr.*RKem.*RKa./RV). * ...
[exp(-RKr.*Tc)./((RKem-RKr).*(RKa-RKr).*RKel-RKr)) + ...
exp(-RKem.*Tc)./((RKr-RKem).*(RKa-RKr).*(RKel-RKem)) + ...
exp(-RKa.*Tc)./((RKr-RKa).*(RKem-RKa).*(RKel-RKa)) + ...
exp(-RKel.*Tc)./((RKr-RKel).*(RKem-RKel).*(RKa-RKel))];

%Calculate final plasma profile
RConc = RIRConc + RECConc;
RConc = RConc .* (1 + randn(length(Ts),N)*ERR_CV); %Introduce residual error.
MATLAB Programming Codes in Fasted Condition (Continued)

% Collect RCmax information

AvgRCmax(trial) = mean(max(RConc));
StdRCmax(trial) = std(max(RConc));
CVRCmax(trial) = StdRCmax(trial)*100/AvgRCmax(trial);

% Simulate plasma profile for test formulation

TKr = ones(length(Ts),1)*KrT; % Prepare parameters for calculation
TKem = ones(length(Ts),1)*Kem(2,:);
TKa = ones(length(Ts),1)*Ka(2,:);
TKel = ones(length(Ts),1)*Kel(2,:);
TV = ones(length(Ts),1)*V(2,:);
Tc = Ts * ones(1,N); % Generate Ts for each N subject

% Calculate plasma profile from IR dose

TIRConc = [IRDose*TKa./TV./(TKa-TKel)] .* (exp(-TKel.*Tc)-exp(-TKa.*Tc));

Tc = Tc - ones(length(Ts),1)*Lag(2,:); % Subtract Lag from Ts for each N subject

Tc(Tc < 0) = 0;
MATLAB Programming Codes in Fasted Condition (Continued)

%Calculate plasma profile from EC dose

\[ TECConc = \left( ECDose \times TKr \times TKem \times TKa / TV \right) \times \left\{ \frac{\exp(-TKr \times Tc)}{(TKem-TKr) \times (TKa-TKr) \times (TKel-TKr)} + \cdots \right\} \]
\[ \frac{\exp(-TKem \times Tc)}{(TKr-TKem) \times (TKa-TKem) \times (TKel-TKem)} + \cdots \]
\[ \frac{\exp(-TKa \times Tc)}{(TKr-TKa) \times (TKem-TKa) \times (TKel-TKa)} + \cdots \]
\[ \frac{\exp(-TKel \times Tc)}{(TKr-TKel) \times (TKem-TKel) \times (TKa-TKel)} \] \]

%Calculate final plasma profile

\[ TConc = TIRConc + TECConc; \]
\[ TConc = TConc \times \left( 1 + \text{randn(length(Ts),N)} \times ERR\_CV \right); \]
%Introduce residual error.

%BE analysis

\[ LNCmax = \log(\max(RConc) \times \max(TConc)); \]
%Create log of Cmax data
%entry

Subject = [1:N 1:N]; %Create 'Subject' data entry
Formulation = [ones(1,N) 2*ones(1,N)]; %Create 'Formulation' data entry
[p,table,stats] = anovan(LNCmax, {Subject, Formulation}, 1, 3, ...}
{Subject', 'Formulation'}, 'off'); %ANOVA of LNCmax data
MSE = stats.mse;
MATLAB Programming Codes in Fasted Condition (Continued)

DF = stats.dfe;
AvgRLNCmax = mean(log(max(RConc)));
AvgTLNCmax = mean(log(max(TConc)));
AvgLNCmaxDiff(trial) = AvgTLNCmax - AvgRLNCmax;
EBar(trial) = tinv(0.9, DF)*sqrt(2*MSE/N);
CI90_U(trial) = AvgLNCmaxDiff(trial) + EBar(trial);
CI90_L(trial) = AvgLNCmaxDiff(trial) - EBar(trial);

if exp(CI90_U(trial)) <= 1.25 & exp(CI90_L(trial)) >= 0.8,
        Decision(trial) = 1;
else
        Decision(trial) = 0;
end

disp('simulation completed."

P_PASS_FASTED = sum(Decision)/ntrials %Probability of passing BE test
MATLAB Programming Codes for Simulations of Bioequivalence Studies in Fed Condition

%This M-Script contains MATLAB commands for performing simulation of %bioequivalent study in FED condition of
% -reference formulation: Adderall XR
% -test formulation: Mixed IR and enteric-coated pellet of amphetamine
%By Prapoch Watanalumlerd (Keng) November 18, 2003

clear all

%Number of simulated BE trials
ntrials = input('Number of trials? ');
disp('start simulation...')
randn('state',sum(100*clock)) %Reset RNG's state

%Define variables
Ts = [0; 1; 2; 3; 4; 5; 6; 7; 8; 9; 10; 11; 12; 14; 16; 24]; %Sampling time
N = 24; %Number of subjects in BE study
Decision = zeros(ntrials, 1); %Pass/Failure decision storage
AvgLNCmaxDiff = zeros(ntrials, 1); %Difference btw. average LNCmax of test
%and ref.
MATLAB Programming Codes in Fed Condition (Continued)

CI90_U = zeros(ntrials, 1); %Upper 90% CI bound
CI90_L = zeros(ntrials, 1); %Lower 90% CI bound
EBar = zeros(ntrials, 1); %tinv(0.9, DF)*sqrt(2*MSE/N) storage

%Variables for ref. Cmax analysis
AvgRCmax = zeros(ntrials, 1); %Ref. average Cmax from each trial
StdRCmax = zeros(ntrials, 1); %Ref. Std. of Cmax from each trial
CVRCmax = zeros(ntrials, 1); %Ref. %CV of Cmax from each trial

%Define parameters
IRDose = 4688; %IR dose (in mcg) equivalent to 10000 mcg salts
ECDose = 4688; %EC dose (in mcg) equivalent to 10000 mcg salts
KrR_mu = 3.5; %Mean value for drug release rate constant (EC ref. dose) in pH 6.0 buffer
KrR_ERR_CV = 0.05; %Residual CV for KrR
KrT_mu = 0.693; %Mean value for release rate constant (EC test dose) in pH 6.0 buffer
KrT_ERR_CV = 0.05; %Residual CV for KrT
Lag_mu = 1; %Population mean value for lag time of emptying of pellets in fed %state
MATLAB Programming Codes in Fed Condition (Continued)

Lag_ETA_CV = 0.5; %Lag CV-intersubject
Lag_EPS_CV = 0.3; %Lag CV-intrasubject
GET_mu = 5.7; %Population mean value for gastric emptying time of pellets in fed
%state

GET_ETA_CV = 0.5; %GET CV-intersubject
GET_EPS_CV = 0.3; %GET CV-intrasubject
Ka_mu = 0.744; %Population mean value for Ka
Ka_ETA_CV = 0.25; %Ka CV-intersubject
Ka_EPS_CV = 0.15; %Ka CV-intrasubject
Kel_mu = 0.067; %Population mean value for Kel
Kel_ETA_CV = 0.25; %Kel CV-intersubject
Kel_EPS_CV = 0.15; %Kel CV-intrasubject
V_mu = 247; %Population mean value for V (in L)
V_ETA_CV = 0.25; %V CV-intersubject
V_EPS_CV = 0.15; %V CV-intrasubject
ERR_CV = 0.05; %Residual (assay and model misspecification) CV

%Do the simulation ntrials times

for trial = 1:ntrials,
    %Counter
MATLAB Programming Codes in Fed Condition (Continued)

if rem(ntrials - trial, 200) == 0,
    disp(ntrials - trial)
end

%Generate PK parameters
KrR = KrR_mu * exp(randn(1,N)*KrR_ERR_CV); %Introduce residual var. (Ref.)
KrT = KrT_mu * exp(randn(1,N)*KrT_ERR_CV); %Introduce residual var. (Test)
Lag = ones(2,1) * Lag_mu * exp(randn(1,N)*Lag_ETA_CV); %Introduce interindividual var.
Lag = Lag .* exp(randn(2,N)*Lag_EPS_CV); %Introduce intraindividual var.
GET = ones(2,1) * GET_mu * exp(randn(1,N)*GET_ETA_CV); %Introduce interindividual var.
GET = GET .* exp(randn(2,N)*GET_EPS_CV); %Introduce intraindividual var.
K0 = ECDose / GET; %Generate K0
Ka = ones(2,1) * Ka_mu * exp(randn(1,N)*Ka_ETA_CV); %Introduce interindividual var.
MATLAB Programming Codes in Fed Condition (Continued)

\[ Ka = Ka \cdot \exp(\text{randn}(2,N) \cdot Ka_{\text{EPS.CV}}); \% \text{Introduce intraindividual var.} \]

\[ Kel = \text{ones}(2,1) \cdot Kel_{\mu} \cdot \exp(\text{randn}(1,N) \cdot Kel_{\text{ETA.CV}}); \% \text{Introduce interindividual var.} \]

\[ Kel = Kel \cdot \exp(\text{randn}(2,N) \cdot Kel_{\text{EPS.CV}}); \% \text{Introduce intraindividual var.} \]

\[ V = \text{ones}(2,1) \cdot V_{\mu} \cdot \exp(\text{randn}(1,N) \cdot V_{\text{ETA.CV}}); \% \text{Introduce interindividual var.} \]

\[ V = V \cdot \exp(\text{randn}(2,N) \cdot V_{\text{EPS.CV}}); \% \text{Introduce intraindividual var.} \]

\% Simulate plasma profile for reference formulation

\[ RK_r = \text{ones}(\text{length}(Ts),1) \cdot KrR; \% \text{Prepare parameters for calculation} \]

\[ RK_0 = \text{ones}(\text{length}(Ts),1) \cdot K0(1,:); \]

\[ RGET = \text{ones}(\text{length}(Ts),1) \cdot \text{GET}(1,:); \]

\[ RK_a = \text{ones}(\text{length}(Ts),1) \cdot Ka(1,:); \]

\[ RK_{el} = \text{ones}(\text{length}(Ts),1) \cdot Kel(1,:); \]

\[ RV = \text{ones}(\text{length}(Ts),1) \cdot V(1,:); \]

\[ T_c = Ts \times \text{ones}(1,N); \% \text{Generate Ts for each N subject} \]
MATLAB Programming Codes in Fed Condition (Continued)

%Calculate plasma profile from IR dose
\[
RIRConc = \left[ \text{IRDose} \cdot \frac{RKa}{RV} \cdot \frac{1/(RKa-RKe)}{\exp(RKe1 \cdot Tc)} - \exp(-RKe1 \cdot Tc) \right] \cdot \left( \exp(RKe1 \cdot Tc) \cdot \exp(RKa \cdot Tc) \right);
\]

\[
Tc = Tc - \text{ones}(\text{length}(Ts),1) \cdot \text{Lag}(1,:); \quad \text{subtract Lag from Ts for each N subject}
\]

\[
Tc(Tc < 0) = 0;
\]

\[
Tg = Tc - \text{RGET}; \quad \text{subtract GET from Tc for each N subject}
\]

\[
Tg(Tg < 0) = 0;
\]

%Calculate plasma profile from EC dose

\[
TcA = Tc \geq \text{RGET}; \quad \text{set Tc before GET to zero}
\]

\[
TcB = Tc < \text{RGET}; \quad \text{set Tc after GET to zero}
\]

%T < GET

\[
\text{REC} = TcB \cdot \left[ (RKe1/(RV \cdot RKe)) \cdot \left( 1 - \exp(-RKa \cdot Tc) \right) / ((RKa-RKr) \cdot (RKa-RKe)) - \exp(-RKa \cdot Tc) / ((RKa-RKr) \cdot (RKa-RKe)) - \exp(-RKe1 \cdot Tc) / ((RKa-RKr) \cdot (RKa-RKe)) - \exp(-RKa \cdot Tc) / ((RKa-RKr) \cdot (RKa-RKe)) \right];
\]
MATLAB Programming Codes in Fed Condition (Continued)

%T > GET

\[
RC1 = TcA \cdot (RKO ./ (RV \cdot RKe1)) \cdot [1 - (RKa \cdot RKe1 \cdot \exp(-RKr \cdot RGET) \cdot (RKe1 - RKr) - RKe1 \cdot \exp(-RKr \cdot Tg)]
\]

\[
RC2 = TcA \cdot (((RKa \cdot RKe1 \cdot \exp(-RKr \cdot RGET) \cdot (RKe1 - RKr) - RKe1 \cdot \exp(-RKr \cdot Tg)) + (RKa \cdot \exp(-RKr \cdot Tg) \cdot (RKe1 \cdot \exp(-RKr \cdot Tg))))
\]

\[
RC3 = TcA \cdot (((RKa \cdot RKe1 \cdot \exp(-RKr \cdot RGET) \cdot (RKe1 - RKr) - RKe1 \cdot \exp(-RKr \cdot Tg)) + (RKa \cdot \exp(-RKr \cdot Tg) - RKe1 \cdot \exp(-RKr \cdot Tg)))
\]

\[
RECConc = REC + RC1 + RC2 + RC3; \text{ %Plasma profile from EC Dose}
\]
MATLAB Programming Codes in Fed Condition (Continued)

%Calculate final plasma profile
RConc = RIRConc + RECCconc;
RConc = RConc .* (1 + randn(length(Ts),N)*ERR_CV); %Introduce %residual error.

%Collect RCmax information
AvgRCmax(trial) = mean(max(RConc));
StdRCmax(trial) = std(max(RConc));
CVRCmax(trial) = StdRCmax(trial)*100/AvgRCmax(trial);

%Simulate plasma profile for test formulation
TKr = ones(length(Ts),1)*KrT; %Prepare parameters for calculation
TK0 = ones(length(Ts),1)*K0(2,:);
TGET = ones(length(Ts),1)*GET(2,:);
TKa = ones(length(Ts),1)*Ka(2,:);
TKel = ones(length(Ts),1)*Kel(2,:);
TV = ones(length(Ts),1)*V(2,:);

Tc = Ts * ones(1,N); %Generate Ts for each N subject
% Calculate plasma profile from IR dose

\[ \text{TIRConc} = [\text{IRDose} \times \text{TKa}/\text{TV}/(\text{TKa} - \text{TKel})] \times (\exp(-\text{TKel} \times \text{Tc}) - \exp(-\text{TKa} \times \text{Tc})); \]

\[ \text{Tc} = \text{Tc} - \text{ones(length(Ts),1)} \times \text{Lag(2,:)}; \% \text{Subtract Lag from Ts for each N subject} \]

\[ \text{Tc}(\text{Tc} < 0) = 0; \]

\[ \text{Tg} = \text{Tc} - \text{TGET}; \% \text{Subtract GET from Tc for each N subject} \]

\[ \text{Tg}(\text{Tg} < 0) = 0; \]

% Calculate plasma profile from EC dose

\[ \text{TcA} = \text{Tc} \geq \text{TGET}; \% [0 \ 0 \ 0 \ 1 \ 1 \ 1] \ -- \text{set Tc before GET to zero} \]

\[ \text{TcB} = \text{Tc} < \text{TGET}; \% [1 \ 1 \ 1 \ 0 \ 0 \ 0] \ -- \text{set Tc after GET to zero} \]

% T < GET

\[ \text{TEC} = \text{TcB} \times ((\text{TK0}/(\text{TV} \times \text{TKel}))) \times ... \]

\[ [1-(\text{TKa} \times \text{TKel} \times \exp(-\text{TKr} \times \text{Tc}))/((\text{TKa} - \text{TKr}) \times (\text{TKel} - \text{TKr})) - ... \]

\[ (\text{TKr} \times \text{TKel} \times \exp(-\text{TKa} \times \text{Tc}))/((\text{TKr} - \text{TKa}) \times (\text{TKel} - \text{TKa})) - ... \]

\[ (\text{TKa} \times \text{TKr} \times \exp(-\text{TKel} \times \text{Tc}))/((\text{TKr} - \text{TKel}) \times (\text{TKa} - \text{TKel}))]; \]
MATLAB Programming Codes in Fed Condition (Continued)

%T > GET

TC1 = TcA .* (TK0./(TV.*TKel)).* ...

[1-(TKa.*TKel.*exp(-TKr.*TGET))./((TKa-TKr).*(TKel-TKr))- ...
(TKr.*TKel.*exp(-TKa.*TGET))./((TKr-TKa).*(TKel-TKa))- ...
(TKa.*TKr.*exp(-TKel.*TGET))./((TKr-TKe1).*(TKa-TKe1))).*exp(-
TKel.*Tg);

TC2 = TcA .* ((TKa.*TK0.*(1-exp(-TKr.*TGET)))./TV).* ...

(exp(-TKr.*Tg)./((TKa-TKr).*(TKel-TKr)) + ...
exp(-TKa.*Tg)./((TKr-TKa).*(TKel-TKa)) + ...
exp(-TKel.*Tg)./((TKr-TKe1).*(TKa-TKe1)));

TC3 = TcA .* ((TKa.*TK0.*TKr)./(TV.*(TKa-TKe1))).* ...

[1./(TKr.*TKa)+exp(-TKr.*TGET)./(TKr.*(TKr-TKa)) + ...
exp(-TKa.*TGET)./(TKa.*(TKa-TKr))].* ...
(exp(-TKel.*Tg)-exp(-TKa.*Tg));

TECCconc = TEC + TC1 + TC2 + TC3; %Plasma profile from EC Dose
MATLAB Programming Codes in Fed Condition (Continued)

%Calculate final plasma profile

TConc = TIRConc + TECCconc;

TConc = TConc .* (1 + randn(length(Ts),N)*ERR_CV); %Introduce
%residual error.

%BE analysis

LNCmax = log([max(RConc) max(TConc)]); %Create log of Cmax data
%
%entry

Subject = [1:N 1:N]; %Create 'Subject' data entry

Formulation = [ones(1,N) 2*ones(1,N)]; %Create 'Formulation' data entry

[p,table,stats] = anovan(LNCmax, {Subject, Formulation}, 1, 3,
{'Subject' ; 'Formulation'}, 'off'); %ANOVA of LNCmax data

MSE = stats.mse;

DF = stats.dfe;

AvgRLNCmax = mean(log(max(RConc)));

AvgTLNCmax = mean(log(max(TConc)));

AvgLNCmaxDiff(trial) = AvgTLNCmax - AvgRLNCmax;

EBar(trial) = tinv(0.9, DF)*sqrt(2*M5EIN);

C190_U(trial) = AvgLNCmaxDiff(trial) + EBar(trial);

C190_L(trial) = AvgLNCmaxDiff(trial) - EBar(trial);
MATLAB Programming Codes in Fed Condition (Continued)

    if exp(C190_U(trial)) <= 1.25 & exp(C190_L(trial)) >= 0.8,
    Decision(trial) = 1;
    else
    Decision(trial) = 0;
    end

end

disp('simulation completed.')

P_PASS_FED = sum(Decision)/ntrials %Probability of passing BE test
Appendix 6

Derivation of Pharmacokinetic Models for Leaky Enteric-Coated Pellets in Fasted Condition

Figure A.11  Compartmental diagram of pharmacokinetic model for leaky enteric-coated pellets in fasted condition. \( X_{PS} \), amount of drug in pellets form in the stomach; \( X_{SS} \), amount of dissolved drug in the stomach; \( X_{SI} \), amount of dissolved drug in the intestine; \( X_1 \), amount of drug in plasma/blood; \textbf{Dose}, a leaky enteric-coated dose; \( k_{em} \), a first-order rate of drug input into the intestine corresponding to the first-order gastric emptying of pellets in fasted condition; \( k_r \), a first-order release rate of drug from pellets within the stomach; \( k_s \), a first-order rate of drug input into the intestine corresponding to the first-order gastric emptying of liquid; \( k_a \), a first-order absorption rate constant of drug; \( k_{el} \), a first-order elimination rate constant of drug.

Four differential equations describing compartmental scheme above are

\[
\frac{dX_{PS}}{dt} = -k_{em}X_{PS} - k_rX_{PS}
\]  
A.6.1
\[
\frac{dX_{SS}}{dt} = k_r X_{PS} - k_s X_{SS} \quad \text{A.6.2}
\]

\[
\frac{dX_{SI}}{dt} = k_{em} X_{PS} - k_s X_{SS} - k_a X_{SI} \quad \text{A.6.3}
\]

\[
\frac{dX_1}{dt} = k_a X_{SI} - k_{el} X_1 \quad \text{A.6.4}
\]

Laplace transform of equation 3.17 and rearrange

\[
\bar{X}_{PS} = \frac{D}{s + k_{em} + k_r} \quad \text{A.6.5}
\]

Let \( k_c = k_{em} + k_r \)

Take antiLaplace

\[
X_{PS} = D \cdot e^{-k_c t} \quad \text{A.6.6}
\]

Substitute equation A.2.6 into equation 3.18

\[
\frac{dX_{SS}}{dt} = k_t D \cdot e^{-k_c t} - k_s X_{SS} \quad \text{A.6.7}
\]

Laplace transform of equation A.2.7 and rearrange

\[
\bar{X}_{SS} = \frac{k_t D}{(s + k_s)(s + k_c)} \quad \text{A.6.8}
\]

Take antiLaplace

\[
X_{SS} = \frac{k_t D}{(k_c - k_s)} \left[ e^{-k_s t} - e^{-k_c t} \right] \quad \text{A.6.9}
\]

Substitute equation A.2.9 into equation 3.19

\[
\frac{dX_{SI}}{dt} = k_{em} D \cdot e^{-k_c t} + \frac{k_t k_s D}{(k_c - k_s)} \left[ e^{-k_s t} - e^{-k_c t} \right] - k_a X_{SI} \quad \text{A.6.10}
\]
Laplace transform of equation A.2.10 and rearrange

$$\bar{X}_{SI} = \frac{k_{em}Ds + k_c k_s D}{(s + k_c)(s + k_s)(s + k_a)}$$  \hspace{1cm} A.6.11

Take antiLaplace

$$X_{SI} = \frac{(-k_{em}k_a D + k_c k_s D)e^{-k_a t}}{(k_s - k_a)(k_c - k_a)} + \frac{(-k_{em}k_s D + k_c k_c D)e^{-k_s t}}{(k_a - k_s)(k_c - k_c)}$$

$$+ \frac{(-k_{em}k_c D + k_c k_s D)e^{-k_c t}}{(k_a - k_c)(k_s - k_c)}$$  \hspace{1cm} A.6.12

Substitute equation A.2.12 into equation 3.20

Laplace transform and rearrange

$$\bar{X}_I = \frac{k_{em}k_aDs + k_c k_s k_a D}{(s + k_a)(s + k_s)(s + k_c)(s + k_{cl})}$$  \hspace{1cm} A.6.13

Take antiLaplace

$$X_I = k_a D \left[ \frac{(k_c k_s - k_{em} k_c) e^{-k_c t}}{(k_a - k_c)(k_s - k_c)(k_{cl} - k_c)} + \frac{(k_c k_s - k_{em} k_s) e^{-k_s t}}{(k_a - k_s)(k_c - k_s)(k_{cl} - k_s)} + \frac{(k_c k_s - k_{em} k_a) e^{-k_a t}}{(k_s - k_a)(k_c - k_a)(k_{cl} - k_a)} \right]$$

$$+ \frac{(k_c k_s - k_{em} k_{cl}) e^{-k_{cl} t}}{(k_a - k_{cl})(k_s - k_{cl})(k_c - k_{cl})}$$  \hspace{1cm} A.6.14
\[ C_1 = \frac{k_a D}{V} \left[ \frac{(k_c k_s - k_{em} k_c) e^{-k_s t}}{(k_a - k_c)(k_s - k_c)(k_{cl} - k_c)} + \frac{(k_c k_s - k_{em} k_s) e^{-k_s t}}{(k_a - k_s)(k_c - k_s)(k_{cl} - k_s)} + \frac{(k_c k_s - k_{em} k_a) e^{-k_s t}}{(k_s - k_a)(k_c - k_a)(k_{cl} - k_a)} \right. \\
\left. + \frac{(k_c k_s - k_{em} k_{cl}) e^{-k_s t}}{(k_a - k_{cl})(k_s - k_{cl})(k_c - k_{cl})} \right] \]

A.6.15
%This M-Script contains MATLAB commands that simulate one plasma concentration time profile from a "leaky coat" formulation of RIBOFLAVIN.

%-The drug release from beads in the stomach follows FIRST order process.
%-Gastric emptying rate of beads is that from fed stomach (zero order).
%-Drug release is instantaneous once beads enter the intestine.

%By Prapoch Watanalumlerd (Keng) February 2003

%Define parameters' value
Dose = 60000;  %Dose given (mcg)
T = 12;  %Last input and plotting time
Tinc = 0.1;  %Input time increment
Bead = 200;  %Amount of beads in dose given
Kr = 0.144;  %First order drug release rate constant from bead in the stomach
KrRM = exp(-Kr*Tinc);  %Fraction of drug remained in bead after Tinc has passed

Example of MATLAB Programming Codes (Continued)
GET = 5.7;  %Gastric emptying time of beads
K0 = 1/GET;  %Zero order gastric emptying rate
BEmpty = Bead*K0;  %Zero order emptying rate of beads
T50 = 0.25;  %Half time of emptying of liquid
Kem = 0.693/T50;  %Emptying rate constant of soluble drug from the stomach
KemRM = exp(-Kem*Tinc);  %Fraction of soluble drug remained in the stomach after Tinc has passed
DPB = Dose/Bead;  %Dose per bead at current time
F = 0.85;  %Fraction of drug absorbed
Ka = 3.67;  %First order absorption rate constant
Kel = 0.32;  %First order elimination rate constant
V = 190.7;  %Volume of distribution

%Check validity of inputs

if mod(T, Tinc) ~= 0,
    error('Modulus after division of T by Tinc is not zero. Change "T" or "Tinc".')
end
Example of MATLAB Programming Codes (Continued)

%Initial variables
STdrug = 0;  %Released drug in the stomach
TTSTdrug = 0;  %Total released drug in the stomach
DEmpty = 0;  %Soluble drug emptied from the stomach
RSTdrug = 0;  %Soluble drug remained in the stomach
DIntbead = 0;  %Drug entering the intestine via beads

%Do the simulation

for i = Tinc : Tinc : T,
    %Calculate the amount of drug input from soluble drug in the stomach
    STdrug = max(0, (Bead - BEmpty * (i - Tinc)) * DPB * (1 - KrRM));
    TTSTdrug = STdrug + RSTdrug;
    DEmpty = [DEmpty; ((1 - KemRM) * TTSTdrug)];
    RSTdrug = TTSTdrug * KemRM;

    %Update DPB
    if i <= GET,
        DPB = DPB * KrRM;  %Adjust DPB as drug is released from beads
    else
        DPB = DPB * KrRM;
    end
end
Example of MATLAB Programming Codes (Continued)

```
DPB = 0; % when time >= emptying time, set DPB = 0
end

% Calculate the amount of drug entering the intestine via beads
DIntbead = [DIntbead; (DPB * BEmpty * Tinc)];
end

% Calculate the drug input for the blood compartment
DInput = DEmpty + DIntbead;

TPlot = [0 : Tinc : T]; % Create time parameter for the plot
z = T/Tinc + 1;
DConc = zeros(1, z); % Create "DConc" variable to store the
                            % output concentration from each input

for j = Tinc : Tinc : T,
    % Adjust the "TPlot"
    AdjTPlot = TPlot - j;
```
Example of MATLAB Programming Codes (Continued)

%Replace any values in "AdjTPlot" that less than 0 with 0
AdjTPlot(AdjTPlot < 0) = 0;

%Calculate blood concentration produced by input from j
k = round(j/Tinc);
Conc = (F * DInput(k+1) * Ka / (V * (Ka - Kel))) * ...
    (exp(-Kel * AdjTPlot) - exp(-Ka * AdjTPlot));

%Store the output in "DConc"
DConc = [DConc; Conc];
end

SMDConc = sum(DConc);
Cmax = max(SMDConc);
figure
plot(TPlot,SMDConc,':')