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INTEGRATION OF DISSOLUTION TECHNOLOGY WITH COMPUTER SIMULATIONS TO PREDICT ORAL DRUG ABSORPTION

by

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ABSTRACT

The objectives of this study were to develop dissolution test methods that can be used to predict the oral absorption of two drug products, etoricoxib (Arcoxia®) and montelukast sodium (Singulair®), and to assess the capability of computer simulations using GastroPlus™ to establish *in vitro/ in vivo* correlations (IVIVC).

Drug solubility was measured in different media, and the dissolution behaviour of the drug products were studied in the USP Apparatus 2 using the USP buffers and biorelevant dissolution media (BDM) and in the flow-through cells following dynamic pH change protocols. Drug permeability was assessed *in vitro* using the Caco-2 and MDCK cell culture techniques.

Simulations indicated that montelukast sodium bioavailability is dissolution rate limited, while that of etoricoxib is neither permeability nor dissolution rate limited. Physiologically based computer simulation models using *in vitro* data are a promising tool in predicting the *in vivo* behaviour of oral drug products and for establishing IVIVC.

DEDICATION

This work is dedicated to my beloved children Angelous Lakeisha Ginanena and Ester-Angela Acaye Ginanena who had to spend a good 2 years of their early life without daddy and to my wife Levina, without whose support, this work would not have been possible.

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Arthur G Okumu, 2007.

"If you are neutral in situations of injustice, you have chosen the side of the oppressor. If an elephant has its foot on the tail of a mouse and you say that you are neutral, the mouse will not appreciate your neutrality." Bishop Desmond Tutu.

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ABREVIATIONS

ACAT	Advanced Compartmental Absorption and Transit
ADMET	Absorption Distribution Metabolism Excretion Toxicity
AIC	Akaike Information Criterion
An	Absorption Number
API	Active Pharmaceutical Ingredient
AUC	Area under the Plasma Concentration-Time curve
BCS	Biopharmaceutics Classification System
BDM	Biorelevant Dissolution Media
Cmax	Maximum Plasma Concentration
CMC	Critical Micelle Concentration
CR	Controlled Release
DMEM	Dulbecco's Modified eagle's Medium
Dn	Dissolution Number
Do	Dose Number
EDTA	Ethylenediamine tetraacetic acid
EMEA	European Agency for the Evaluation of Medicinal Products
FaSSIF	Fasted State Simulated Intestinal Fluid
FBS	Fetal Bovine Serum
FDA	The US Food and Drug Administration
FeSSIF	Fed State Simulated Intestinal Fluid
FIP	International Pharmaceutical Federation
GI	Gastro Intestinal
HBSS	Hank's Balanced Salts Solution
HCL	Hydrochloric Acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC	High Performance Liquid Chromatography
IDR	Intrinsic Dissolution Rate
IND	Investigational New Drug

IR	Immediate Release
IV	Intravenous
IVIVC	*In Vitro In Vivo* Correlation
kV	Kilovolts
mA	Milliamperes
MAE	Mean Absolute Error
MDCK	Madin-Darby Canine Kidney
MEM	Minimum Essential Media
mg	Milligrams
mL	Milliliters
mm	Millimeters
mM	Millimolar
NaCl	Sodium Chloride
NDA	New Drug Application
PBS	Phosphate Buffer Saline
PE	Prediction Error
Peff	Effective Permeability
QC	Quality Control
R&D	Research and Development
RPM	Revolutions per Minute
SEM	Scanning Electron Microscopy
SEP	Standard Error of Prediction
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid
SLS	Sodium lauryl Sulphate
SSE	Sums of Squares error
TEER	Trans Epithelial Electrical Resistance
TFA	Trifluoroacetic Acid
USP	United States Pharmacopoeia
UV	Ultra Violet
μM	Micromolar

CHAPTER -1

1 INTRODUCTION

1.1 Background

The oral route is the preferred route of administration for most new pharmaceutical drug products, because it is convenient and economical. There are, however, several factors that can influence oral drug absorption, due to the complexity of the GI tract. The rate and extent to which an orally administered drug is absorbed and reaches systemic circulation depends on various physiochemical, biopharmaceutical, and physiological factors (Dressman et al., 1985; Yu et al., 1996a; Charman et al., 1997; Hörter et al., 1997). The physicochemical factors that have been identified as important for oral drug absorption include partition coefficient, pKa, drug particle surface area, crystal form, solubility of the drug substance in water and physiologically relevant fluids, salt formation, drug diffusion layer pH, particle size, and polymorphism of the drug substance. The biopharmaceutical factors that influence drug permeability include the effects of drug ionization, partition coefficient and polar surface area, and the physiological factors include food effect, presence of bile salts, gastrointestinal transit time, intestinal motility and the gastro intestinal (GI) pH profile.

For orally administered drugs, dissolution rate, solubility in the intestinal fluids and intestinal wall permeability are the key determinants to their oral bioavailability. The various factors that can have an influence on the *in vivo* dissolution and hence bioavailability of an oral solid dosage form are summarized in the Figure 1-1.

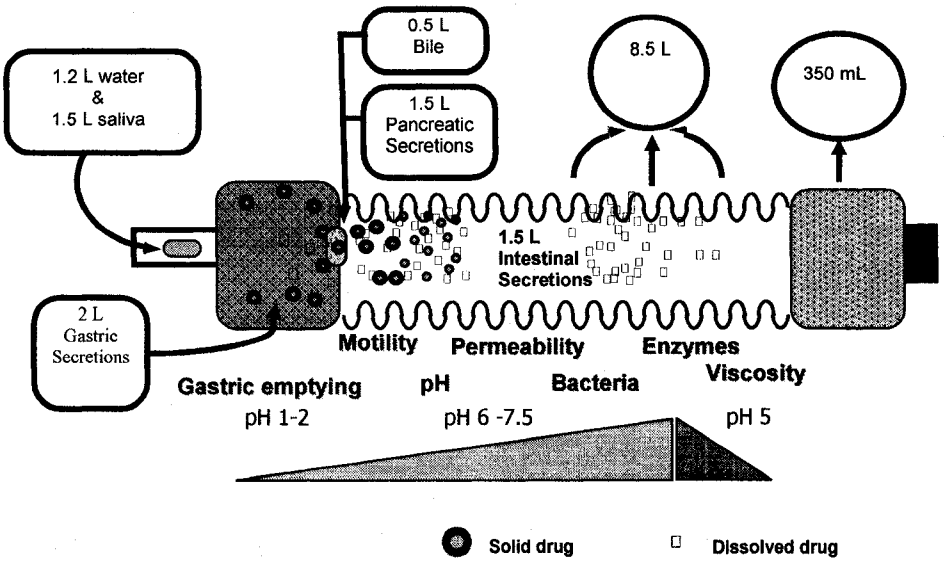

Figure 1-1 Factors influencing the GI drug uptake

The inward arrows and values indicate the volume of fluids consumed and secreted into the GI tract from each of the segments indicated, and the outward flow indicate the volume of fluids re-absorbed in litres, over a 24 h period (Modified from Löbenberg et al., 2000)

Over the past decade, advances in combinatorial chemistry and high throughput screening have dramatically increased the number of drug candidates, most of them with high lipophilicity and many intra-molecular hydrogen bonds (Lipinski et al., 2000, Grass et al., 2002). This property has often resulted into drugs with poor aqueous solubility, leading to variable or poor oral absorption (Takano et al., 2006; Serajuddin, 2002). The overall time and cost of new product development and commercialization (Grass et al., 2002) has, thus, also increased. Dissolution testing is an *in vitro* test used to assess and estimate the *in vivo* behaviour of orally administered solid dosage forms (Dressman et al., 1998), and it has become an industry standard method.

1.2 Dissolution Testing

Dissolution testing in the pharmaceutical industry is used both for quality control (QC) purposes and during drug product development (R&D). As a quality control tool, dissolution testing is used to assess batch to batch conformity, monitor manufacturing processes and may be used to approve changes arising due to scale-up from bio-batch to production batch or to monitor minor changes due to process, formulation or site changes (FDA CDER Guidance to industry, 1997). For R&D purposes, dissolution testing is used to assess the drug release profile and as a formulation screening tool during the drug development process.

The goals of QC and R&D may require the development of two different dissolution protocols (Azarmi et al., 2007). The QC protocol might be intended to detect even small changes that may occur as a result of changes in production method or to demonstrate that the dosage forms were manufactured according to the intended specifications, and that all critical manufacturing steps resulted in a consistent product (Azarmi et al., 2007). For R&D purposes, dissolution tests are intended to be an *in vitro* indicator of the *in vivo* performance of the dosage form (Siewert at al, 2003), and thus the dissolution test method developed should be able to closely mimic the physiological condition the drug is expected to encounter in the GI tract. The dissolution method as an *in-vitro* indicator can also be used as a QC tool to handle changes in manufacturing and allow larger windows for critical processing parameters.

For poorly soluble drugs, dissolution rate *in vivo* might be the rate limiting step in their oral bioavailability. In order to be able to predict the *in vivo* performance, it is critical to develop *in vitro* dissolution test methods which can simulate the *in vivo* behaviour of the drug in the GI tract. The *in vitro* test conditions like the dissolution test medium type, pH, apparatus type and agitation rate may play a significant role in assessing the *in vivo* behaviour (Dressman et al., 1998). The use of surfactants in the dissolution media for testing poorly soluble drugs has been suggested (Shah et al., 1989). Other media that have been used include emulsions and hydroalcoholic or organic solvents (Darwish et al.,

1996; El-Massik et al., 1996). These media however do not reflect the true *in vivo* conditions. Löbenberg et al., (2000) suggested that a more physiologically adapted dissolution media may be necessary if the test is intended as a predictive tool for *in vivo* performance. Thus considering the length of time it takes in drug discovery, it has become important to come up with predictive scientific principles and methods to assess *in vitro*, the *in vivo* performance/behaviour of orally administered drugs early in the development process, in order to minimize costly animal and human studies during or late in the product development process (Li et al., 2005).

There are, however, various fluids including hydrochloric acid and bicarbonate ions that are secreted *in vivo* at different sites and at various rates along the GI tract, adding to the complexity of the composition of the GI contents (Dressman et al., 1998). These luminal contents including bile salts, lecithin and dietary lipids have long been suggested to increase the solubility of poorly soluble drugs and hence improve the dissolution rate and subsequent absorption along the GI tract (Galia et al., 1998, Mithani at al, 1996; Kaukonen et al., 2004)

The regulatory agencies have in recent years placed more emphasis on dissolution testing in the pharmaceutical industry. Dissolution test results can be applied to develop IVIVCs, leading to a reduction in development cost and a faster development process by the reducing the number of costly bioavailability and bioequivalence testing in animals and human subjects (O'Hara et al., 1998). With IVIVC final dissolution specifications are defined to establish similarity between dosage forms for which manufacturing processes, sites or composition have changed within defined limits (FDA, Guidance for Industry, 1995, 1997).

1.3 Dissolution test media

The USP lists examples of different dissolution media used for the dissolution testing of tablets and capsules. Typical media listed include dilute hydrochloric acid, buffers in the physiological pH of 1.0 to 7.5, simulated gastric fluids (with or without enzymes), water

and media with surfactants (with or without acids or buffer) such as polysorbate 80, sodium lauryl sulphate or bile acids (General Chapter <1092>, The USP 29, Suppl.2). These media however only simulate the effect of pH and osmolarity of the media on the drug release, and in the case of surfactant solutions, they are intended to modulate the dissolution rate by increasing the solubility of the drug substances in the aqueous media or by acting as wetting agents (the USP 29).

Galia et al (1998) demonstrated the use of BDM in forecasting trends in the *in vivo* performance of BCS class 1 and class 2 immediate release drug products. They reported that the dissolution behaviour of class 2 drugs were dependent on the choice of the media, while that of class 1 drugs were not affected by the media used. Nicolaides et al., (1998) investigated the usefulness of BDM in predicting food and formulation effect on 4 poorly soluble lipophilic drugs. Results from the study indicated that the use of BDM is a promising *in vitro* tool in forecasting the *in vivo* performance of poorly soluble drugs. Löbenberg et al., (2000) and Kostewicz et al., (2002) reported the usefulness of BDM in predicting *in vivo* behaviour of poorly soluble drugs. The composition of BDM has been published in the International Pharmaceutical Federation (FIP) guidelines, as the Fasted State Simulated Intestinal Fluid (FaSSIF) and the Fed State Simulate Intestinal Fluid (FeSSIF) (Aiache et al., 1997).

Galia et al., (1998) and Shah et al., (1989) have demonstrated that increasing the concentration of bile salts in dissolution media increases the *in vitro* dissolution of many poorly soluble drugs. Increase in solubility is thought to be accomplished either by micellar solubilization at concentrations above the critical micelle concentration or by increasing the wettability of the drug particles (Galia et al., 1998,; Shah et al 1989). Kalantzi et al., (2006) demonstrated that the concentration of bile salts and lecithin increases considerably *in vivo* following the ingestion of food. The ability of bile salts to solubilize drugs has been suggested to depend mainly on the lipophilicity of the drug, with more highly lipophilic drugs showing a marked improvement in solubility compared with less lipophilic drugs (Naylor et al., 1995).

Table 1-2 shows the composition of the BDM that have been used and reported in the literature (Galia et al., 1998.)

Table 1-1 Physicochemical property and composition BDM

(Modified from Galia et al., 1998)

Fasted State Simulated intestinal Fluid		Fed State Simulated intestinal Fluid	
Component	Conc.(mM)	Component	Conc(mM)
Sodium taurocholate	3	Sodium taurocholate	15
Lecithin	0.75	Lecithin	3.75
Sodium dihydrogen phosphate	26.66	Acetic Acid	144
Sodium hydroxide	~13.8	Sodium hydroxide	~101
Sodium Chloride	106	Sodium Chloride	173
Osmolarity (mOsmol kg^{-1})	~270±10	Osmolarity (mOsmol kg^{-1})	~635±10

1.4 Dissolution test apparatus

The USP chapter <711> "dissolution tests" and chapter <724> "drug release" describes *in vitro* dissolution test methods used to assure batch to batch conformity and performance assessments of solid dosage forms (The USP 29, 2006). The pharmacopoeia lists several different dissolution apparatuses: apparatus 1(the rotating basket), apparatus 2 (paddle), apparatus 3 (reciprocating cylinder), apparatus 4 (the flow through cell), apparatus 5 (paddle over disk), apparatus 6 (Cylinder) and apparatus 7 (reciprocating cylinder).

Apparatus 1 and 2 are most commonly used simply because of their ease of set-up and operation (Löbenberg, 2000b). Apparatus 1 and 2 are simple, robust and adequately standardized apparatuses which are used in the pharmaceutical industry all around the world. They are recommended in various guidelines as the first choice for *in vitro* dissolution testing of immediate as well as controlled/modified release dosage forms (FIP guidelines, 1995). Due to the "single container" nature of the paddle/basket apparatus,

experimental difficulties may arise in terms of the need of a change in pH or of any other change in the test medium during an investigation, especially for poorly soluble drugs (FIP guidelines, 1995). As a result, proposals have been made and adopted in the pharmacopeia to increase solubility and dissolution rate by addition of an appropriate amount of surfactant.

The USP apparatus 3 was incorporated into the USP in 1991 (The USP XX11/NF XVII. Supplement 4), and its development was based on the recognition of the need to establish *in vitro* and *in vivo* correlations and the fact that the dissolution results obtained with the USP apparatuses 1 and 2 may be significantly affected by shaft wobble, location, centering, and coning at the bottom of the vessels (Borst et al., 1997, Yu et al., 2002; Qureshi et al., 2003). The USP apparatus 3 is primarily designed for the release testing of extended-release (ER) products, although it has been used for the dissolution testing of immediate release (IR) products containing highly soluble drugs, such as metoprolol and ranitidine, and some IR products of poorly soluble drugs, such as acyclovir (Yu et al., 2002). It offers the advantages of avoiding cone formation and mimicking the changes in physiological conditions and mechanical forces experienced by drug products in the GI tract (Yu et al., 2002).

The USP apparatus 4 (The flow through cell method) was first recommended by the FIP dissolution test working group in 1981 as an alternative to in vitro drug release testing (Langenbucher at al, 1989) and incorporated into the USP in the 1990's. With the flow-through cell, the test specimen is placed in a small cell which is continuously flushed with a stream of dissolution medium, simultaneously providing fresh medium and the mechanical agitation for dissolution of the drug substance. The USP describes two different sizes of cells; the large cell has an internal diameter of 22.6 mm and the small cell has an internal diameter of 12 mm. Cell diameter have been associated with flow rates and hydrodynamic agitation of the fluid on the specimen. Taking into account the small intestinal transit times, the average estimated axial velocity of 1.3 and 1.5 cm/min under fed and fasted conditions , respectively (Sunesen et al., 2005), have been estimated to correspond with a flow rate of less than 8 mL/min in the USP apparatus 4 (Fotaki and

Reppas, 2005). Additionally, other cell types have been described in the literature for testing powders and granules, implants, suppositories, soft gelatine capsules, ointments and creams (Glantzmann, 2006). The apparatus can be operated as an open system, where fresh medium is delivered into the cells and the eluate can be collected as the entire outflow over the sampling interval, or it can be fractionated and it can be operated as a closed system in which the medium is re-circulated (Brown, 2005). The open system design provides several advantages in some of the difficult cases involving poorly soluble drugs. The ability to change the medium/or pH during a test run facilitates testing the robustness of the formulation with respect to changes in the conditions in the GIT (Fotaki et al., 2005). Other advantages include the possibility of maintaining sink conditions throughout the test, and it has been suggested that it more closely simulate the *in vivo* hydrodynamics compared with the USP paddle or basket apparatus (Qureshi et a, 1994, Perng et al., 2003). The disadvantages of the flow through dissolution method include the possibility of clogging the filter material with insoluble excipients, leading to variable flow rate.

Other apparatus types described include the USP apparatus 5 (paddle over disk), 6 (Cylinder) and 7 (reciprocating cylinder), with their detailed descriptions and use provided in the USP chapter <724> drug release. These are mainly used for the evaluation of drug release from transdermal dosage forms (the USP 29, 2006)

1.5 The concept of Bioavailability

The term bioavailability has been defined as the rate and extent to which the active drug substance is absorbed from the drug product and becomes available at the site of action (the FDA, July 2000). Two types of bioavailability have been defined; relative (or apparent) bioavailability, as the availability of a drug product compared with a recognized standard containing the same active moiety; and absolute bioavailability as the measure of the systemic exposure of an orally administered drug relative to an intravenous dose (Shargel and Yu, 1999). The absolute bioavailability is measured by

determining the blood concentration of the active drug at numerous time points. The area under the concentration-time curve (AUC) is then calculated from the blood or plasma after oral and intravenous (IV) administration. The absolute oral bioavailability can then be estimated using the following equation.

$$\%F = \frac{AUC_{oral} \times Dose_{iv}}{AUC_{iv} \times Dose_{oral}} \times 100 \quad \textbf{Equation 1-1}$$

Where $Dose_{iv}$ and $Dose_{oral}$ are the dose of the intravenous and oral drug, respectively; AUC_{iv} and AUC_{oral} are the area under the plasma-concentration time curve calculated after intravenous and oral administration of the drug, respectively. This implies that %F is the area under the curve normalized for the administered dose.

The terms oral bioavailability and absorption are sometimes used interchangeably and incorrectly (Sietsema, 1989), so it is important to distinguish between the two. Sinko (1999) defined absorption as the amount of drug that passes through the intestinal mucosa and enters the portal vein. For drugs administered orally, in order for the fraction of the dose absorbed to reach systemic circulation, it must not only pass through the intestinal mucosa where it is subject to metabolism by enzymes present in the gut wall (e.g. gut wall first-pass extraction), but it has to pass through the liver as well, where it is subject to first-pass extraction, unless absorption is only through the lymphatic route.

Lymph vessels called lacteals are present in the linings of the GI tract. While most nutrients and drugs absorbed by the small intestine are emptied into the portal venous system and into the liver for processing, fats and lipid are absorbed through the lacteals into the lymphatic system. The lymphatic system joins to form the lymphatic ducts which drains into the subclavian veins and empties their contents into the heart through the superior vena cava. These eventually get pumped by the heart into systemic circulation. Drugs absorbed by this route can escape first pass metabolism, because they do get to reach systemic circulation before passing through the liver.

The fraction of dose absorbed and, hence, bioavailability can alternatively be estimated using the following equation:

$$F = f_a \times (1 - f_g) \times (1 - f_h) \qquad \textbf{Equation 1-2}$$

Where f_a is the fraction of the dose that gets absorbed across the apical surface of the enterocytes, f_g and f_h represent the fractions that undergo the gut and the liver (hepatic) extraction, respectively.

Oral bioavailability can therefore be broken down to components that reflect drug delivery into the intestine represented by gastric emptying, intestinal transit, local pH and nutritional status and components reflecting the absorption of drugs from the intestinal lumen represented by dissolution, permeability, particle size, intestinal efflux, carrier mediated transport and first-pass metabolism in the gut and subsequent first-pass hepatic extraction (Hall et al., 1999)

The bioavailability (BA) of an orally administered drug product in a new drug application (NDA), abbreviated new drug application (ANDA) or an investigational new drug application (IND), and how it is measured, is of great concern to the regulatory agencies, e.g. the US food and drug administration (FDA). It is used as a criterion to assess the quality and performance of the drug product *in vivo* (FDA, July 2000). It may also indirectly provide information about the properties of the drug substance before entry into systemic circulation, such as permeability and the influence of pre-systemic enzymes and/or transporters e.g. p-glycoprotein (FDA. July 2002). The bioavailability of an orally administered drug can be affected by several factors, such as formulation excipients that may have an impact on the drug release and subsequent absorption.

1.6 The Biopharmaceutical classification system(BCS)

Amidon et al., (1995) developed a mechanistic approach to predicting oral drug absorption, known as the biopharmaceutical classification systems (BCS). The BCS defines the two fundamental parameters governing the rate and extent of oral drug absorption as solubility and permeability. The BCS places orally administered drugs into four categories based on their aqueous solubility and permeability characteristics. The primary objectives of the BCS was to set up a theoretical basis upon which an *in vitro-in vivo* correlation for oral drug products can be established and to aid in setting dissolution standards that can serve as surrogates for costly *in vivo* bioavailability studies (Amidon et al., 1995). According to the BCS, a drug is considered highly soluble if the highest dose can be completely dissolved in 250 mL or less, of buffer with pH adjusted between the pH 1.2 to 7.5; and a drug is considered highly permeable if its measured human effective permeability is equal to or greater than 2×10^{-4} cm/s (Amidon et al., 1995). Table 1-2 summarises the BCS.

Table 1-2 The biopharmaceutical classification system

(From Amidon et al., 1995)

Class	Solubility	Permeability
I	High	High
II	Low	High
III	High	Low
IV	Low	Low

The BCS is based on a simple absorption model, in which the intestine is considered to be a cylindrical tube from which drug absorption occurs, drug particles are spherical and are of uniform size, no reactions or metabolism takes place in the intestine, solubility is independent of particle size and the intestinal pH gradient, and no aggregation occurs

(Amidon et al., 1995). The model identified the key parameters controlling drug absorption as three dimensionless numbers: - an Absorption number (An), Dissolution numbers (Dn) and a Dose number (Do), representing the fundamental processes of membrane permeation, drug dissolution and dose, respectively (Amidon et al., 1995). The dimensionless numbers are expressed using the equations:

$$An = \frac{P_{eff}}{R} * t_{res} = \frac{t_{res}}{t_{abs}} \qquad \textbf{Equation 1-3}$$

$$Dn = t_{res} * \frac{3DC_s}{\rho r^2} = \frac{t_{res}}{t_{diss}} \qquad \textbf{Equation 1-4}$$

$$Do = \frac{M_o / V_o}{C_s} \qquad \textbf{Equation 1-5}$$

Where M_o is the dose administered, V_o is the initial gastric compartment volume (~ 250 mL), C_s is the saturation solubility, t_{res} is the mean residence time (~180 min), t_{diss} is the time required for the drug particles to dissolve, P_{eff} is the effective permeability, and R is the radius of the intestinal segment.

The BCS, however, addresses only two of the potential four factors limiting oral bioavailability, namely drug solubility and permeability across the epithelia lining the GIT (Dressman, 1998). It does not address issues surrounding drug stability and drug metabolism/decomposition in the GI tract, both of which might have an impact on the rate and extent of oral drug absorption.

Class 1 drugs have a high solubility and high permeability. For this class of drugs according to the BCS, the rate limiting step to drug absorption is drug dissolution or gastric emptying, if dissolution is rapid. No IVIVC is expected between the *in vitro* and *in vivo* dissolution for an immediate release (IR) dosage for a class 1 drug, (Amidon et

al., 1995). The dissolution specification for such a drug would require that at least 85% of the drug enclosed in the dosage form is dissolved in less than 15 minutes to ensure bioequivalence (Amidon et al., 1995)

Class 2 drugs have low solubility and high permeability. For this class of drugs, drug absorption may be limited by the solubility (Do) or by the dissolution rate (Dn), which may lead to an incomplete absorption and bioavailability, although their absorption number (An) may be high (Amidon et al., 1995). These drugs are particularly sensitive to factors that have an influence on the gastric transit time (Martinez et al., 2002). If the Dn <1, then the dissolution process may be too slow to enable the drug to complete dissolution before going past its absorption window. In such a case, a good IVIVC can be expected if the *in vitro* dissolution rate is similar to the *in vivo* dissolution, or if the *in vitro* dissolution medium/method is made to closely mimic the *in vivo* dissolution behaviour (Amidon et al., 1995; Dressman et al., 1998).

Class 3 drugs have a high solubility and low permeability, and so permeability is the rate controlling step in drug absorption. There is no or limited IVIVC expected for this class of drugs. Their *in vitro* behaviour, if dissolution rate is fast, is similar to that of class 1 drugs, and so their absorption is governed by physiological factors in the GI tract rather than dosage form factors (Amidon et al., 1995)

Class 4 drugs preset a significant problem to oral delivery, exhibiting extremely low bioavailability. Both drug solubility/dissolution and permeability are considered to be the rate limiting factors to drug absorption, and therefore no correlation is expected between the *in vitro* and *in vivo* dissolution (Amidon et al., 1995).

1.7 The Concept of *in vitro/ in vivo* correlation (IVIVC)/relationship (IVIVR)

The USP defines IVIVC as "the establishment of a rational relationship between a biological property, or a parameter derived from a biological property produced by a

dosage form, and a physicochemical property or characteristic of the same dosage form"(The USP #29, <1088>,2006). The FDA guidelines define the same concept as "a predictive mathematical model describing the relationship between an *in vitro* property of the dosage form and an *in vivo* response (FDA guidelines, 1997). The FDA guidelines states that the main objective of developing an IVIVC is to enable dissolution testing to serve as a surrogate for *in vivo* bioavailability, enabling the reduction in the number of bioequivalence studies required during scale-up and post approval changes (FDA, 1997). Typically the *in vitro* property sought after is the rate or extent of drug dissolution or release, while the *in vivo* response is the plasma drug concentration. According to the USP, the biological property derived is the AUC or C_{max} while the *in vitro* physicochemical property is the dissolution profile. A comprehensive review on the development and application of IVIVC by pharmaceutical scientists was recently published (Emami 2006).

The 3 levels of IVIVC described in the USP are identified as level A, B and C, and in the FDA guidelines, an additional level called the multiple level C IVIVC is described. Level A is a point to point relationship between the drug release profile *in vitro* and the *in vivo* in put rate. It is considered the highest category of correlation. The *in vitro* dissolution curve and the *in vivo* input rate are either superimposable or can be made to be superimposable by a suitable scaling factor (Brockmeier et al., 1985; FDA, 1997). Normally the correlation is expected to be linear, although a concern has been raised about non-linear relationships, but no formal guidance on non-linear IVIVC has been established (FDA, 1997).

The *in vivo* absorption data used for establishing the correlation is usually obtained by a model independent deconvolution approach (Yu et al., 1997) or using either the Wagner-Nelson or the Loo-Riegleman methods, which are model dependent, followed by comparison of the fraction of drug absorbed with the fraction of drug dissolved (FDA, 1997). Level A correlations has traditionally been the most common IVIVC approach and requires a linear relationship between the fraction of drug absorbed and the fraction of drug dissolved (FDA, 1997). It has been postulated that only drug products with

dissolution rate-limited absorption (and with complete absorption) can be expected to exhibit Level A correlations with a slope of one and an intercept of zero (Polli et al., 1996)

Level B correlation is established between the mean *in vitro* dissolution time and either the mean residence time or the mean *in vivo* dissolution time, using the principles of statistical moment analysis. It is a single point measure of the *in vivo* input rate, although the entire *in vitro* and *in vivo* data is used. Level B correlations do not uniquely reflect the actual *in vivo* plasma level curve, because a number of different *in vivo* curves will produce similar mean residence time values.

A Level C IVIVC establishes a single point relationship between a dissolution parameter, for example, t_{50} (i.e. 50% percent of drug dissolved at a time point *t*) and a pharmacokinetic parameter (e.g. AUC or C_{max}). Similar to level B, a Level C correlation does not reflect the complete shape of the plasma concentration time curve.

A multiple Level C correlation relates one or several pharmacokinetic parameters of interest to the amount of drug dissolved at several time points of the dissolution profile.

Several researchers have attempted to apply numerous approaches in developing IVIVC with varying degrees of success (Brockmeier et al., 1982, 1985; Chan et al., 1987; Drewe et al., 1993, Dunne et al., 1997; Hayashi et al., 1995, Hwang et al., 1993; Katori et al., 1991, Balan et al., 2001). Most of these attempts have however been focussed on controlled/ extended release (CR/ER), rather than IR products. Similarly, the compendia and regulatory guidance have been provided for CR/ER products (the USP; FDA, 1997). IVIVC analysis for IR products have generally been less successful, leading researchers to have a generally low expectation, while at the same time questioning the appropriateness of subjecting IR products to IVIVC analysis (Polli, 2000).

1.8 In vitro methods and computer models to predict oral drug bioavailability

Traditionally the definitions of IVIVC refer to the comparison between the *in vitro* dissolution and the *in vivo* input rate (e.g. the *in vivo* dissolution of the drug from the dosage form) after oral administration of a drug product. These comparisons, however, do not take into account the interplay between the various physiological factors and the physicochemical properties of the drug that can have a profound impact on the *in vivo* input rate. Several research groups have reported the development of models to describe the relationships between the *in vitro* dissolution tests and the *in vivo* absorption/bioavailability of drug candidates. Theoretical and computer based models of dissolution and permeation processes in the GI tract have been developed and are reported to simulate oral absorption of drugs. These have been described as the mixing tank model (Dressman et al., 1986), the mass balance model (Sinko et al., 1991) and the compartmental absorption model (CAT) (Yu et al., 1996a, 1996b, 1999).

Ginski and Polli (1998) reported developing an integrated dissolution/Caco-2 system that yielded predictive dissolution-absorption relationships of metoprolol tartrate, piroxicam and ranitidine hydrochloride formulations that matched the observed clinical data. Souliman et al., (2006) have recently reported using an artificial digestive system (ADS) to develop a level A IVIVC for acetaminophen IR tablet. The novel *in vitro* system is reported to mimic the *in vivo* behaviour of drugs in the gastro intestinal tract in man. Takano et al., (2006) reported the development of a system for computer simulations to predict the fraction of dose absorbed for twelve poorly soluble BCS class II drugs, using a mini scale dissolution test method. The poorly soluble drugs selected covered neutral, free weak acids and free weak bases. The model was based on the assumptions that the drugs were passively absorbed; dissolution and absorption takes place only in the intestine; a 4 h transit time through the intestine; intestinal fluid volume of 600 mL with an effective surface area of ~800 cm^2 and membrane permeability is high enough not to be limited by the unstirred water layer (Takano et al., 2006).

Physiologically based models for the prediction of the gastrointestinal transit and absorption of drugs in humans have received much attention, recently. Willmann et al., (2004) have reported developing a model that uses the intestinal permeability coefficient of a drug and its solubility in the intestinal fluids as well as physiological parameters such as the gastric emptying time and the intestinal transit time, to study the dependency of the fraction dose absorbed of both neutral and ionizable compounds. Cai et al., (2006) have reported on the evaluation of the PK express model for its ability to predict human bioavailability. The PK express model is reported to be a physiologically based model, which takes into account first pass metabolism, and integrates *in vitro* data with an *in silico* physiologically based pharmacokinetic (PBPK) model to predict human bioavailability.

Wei and Löbenberg (2006) reported integrating dissolution testing using biorelevant media with *in silico* simulations using GastroPlus™, as a predictive tool for glyburide, a class 2 drug. The researchers used dissolution data generated using BDM and a dynamic pH change protocol in the USP Apparatus 2; solubility and Caco-2 permeability data as input functions, to establish an IVIVC for glyburide. Sunesen et al., (2005) reported the establishment of an IVIVC for a poorly soluble drug product, danazol, using the commercial software known as PDx-IVIVC (GloboMax® LLC, Slough, Berkshire, UK). The researchers used the flow through dissolution method and biorelevant dissolution test media.

Application of computational technology during drug discovery and development has the potential to decrease the length of time prior to NDA submission, and reduce the number of experimental procedures required for compound selection (Agoram, 2001). Parrott and Lavé (2002) provided a comparative assessment of two commercial software tools that employ physiologically based models in the prediction of intestinal absorption of drugs in humans. The software they assessed were GastroPlus™ (Simulations Plus Inc, Lancaster, CA, USA) and IDEA™ (LION Bioscience, Heidelberg, Germany). Their findings suggested that the two software programs are comparable in their classification rate.

In this study, GastroPlus™ was used to simulate the *in vivo* profiles of two commercially available drug products using data generated *in vitro* as input functions. GastroPlus™ is a computer program that is used to simulate and model the drug absorption process in the GI tract (GastroPlus™ manual, 2006). The mechanistic simulation model underlying Gastro Plus™ is known as the advanced compartmental absorption and transit model (ACAT) (Agoram et al., 2001). The ACAT model, which was developed following the theories of the BCS (GastroPlus™ manual, 2006) is based on the original CAT model described by Yu et al., (1996b). In the ACAT model the small intestine is divided into nine compartments, which is further subdivided into four compartments each with different transit times in each compartment. The stomach and the colon are treated each as a single compartment. The drug release, dissolution, precipitation, absorption, and transit across the compartments are all described by specific integrated or differential mathematical equations. The mathematical models/equations takes into consideration the physicochemical properties of the drug under study, e.g. pKa, solubility, diffusion coefficient and effective permeability; and the physiological variables e.g. pH, transit times, volume, length, enzymes and transporter proteins (influx/efflux) affecting drug absorption (GastroPlus™ manual, 2006).

The major inputs required by GastroPlus™ include the dose, the pH solubility profile of the drug, intestinal permeability, formulation properties (dissolution profile, drug particle size), and pharmacokinetic parameters (Vd, CL, inter-compartmental rate constants, etc.). The outputs obtained include the fraction of oral dose absorbed and the plasma concentration-time profile.

Considering the complexity of the drug absorption process, GastroPlus™ is considered a powerful tool due to its broad functionality, including pharmacokinetic model fitting, optimization for drug specific models and sensitivity analysis (Parrott and Lavé,2002), although the user interface is complex and requires thorough training. It appears therefore that the physiologically based computer programs use several *in vitro* data in an attempt to predict the entire *in vivo* profile of the drugs under study.

1.9 In vitro methods used in assessing drug permeability

A drug administered as a solid dosage form must first disintegrate and dissolve in the gastrointestinal contents, in order for it to gain access to the epithelium lining the GI tract. To reach systemic circulation, the drug must cross the epithelium by either partitioning into the lipid membrane, by passing through the water filled channels or by combining with specific membrane bound carriers. The factors that influence the absorption of orally administered drugs have been identified as the physicochemical properties of the drug and the physiological environment in the GI tract (Dressman, 1998).The intestinal mucosa, therefore, presents a physical and a biochemical barrier, which influences oral bioavailability (Hildago, 2001, Chan et al., 2004). The physical barriers are due to the tight junctions and the lipid character of the cell membrane, and the biochemical component is provided by the actions of the enzymes and the transporter proteins present in the cells (Hildago, 2001).

Several mechanisms that facilitate drug transport across the GI membrane have been proposed including paracellular pathways, transcellular passive diffusion, vesicular transport and carrier mediated transport (Löbenberg et al., 2000; Hidalgo, 2001; Chan et al., 2004). Figure 1-2 summarizes some of the mechanisms that facilitate and/or limit the passage of drugs across the enterocytes.

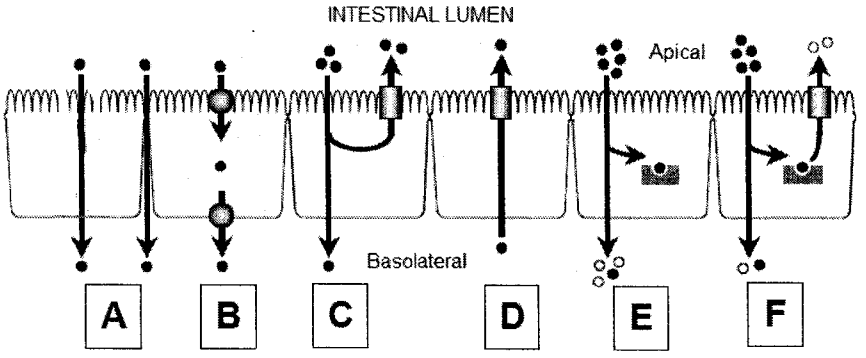

Figure 1-2 The intestinal epithelium: a selective barrier to oral drug absorption.

Where the letters represent:- **A:** passive transcellular and paracellular diffusion; **B:** carrier mediated transport at the apical and/or basolateral membranes; **C:** active efflux transporter on apical membrane may limit absorption; **D:** active efflux transporter on apical membrane, offering an additional route for drug clearance; **E:** intracellular metabolising enzymes localized inside the enterocytes may modify compound, leading to a lower concentration of drug in circulation; **F:** Apical efflux combined with metabolizing enzymes may both form an effective barrier against drug absorption. (Modified from: Chan et al. 2004).

In order to assess the absorption potential of drugs, numerous *in vitro* and *in vivo* models have been reported in literatures. Cell culture models for assessing the intestinal permeability such as, Caco-2 cells (Ranaldi et al., 1992; Luo et al., 2002) and MDCK (Madin-Darby canine kidney) cell lines (Irvine et al., 1999; Braun et al., 2000, 2002; Luo et al., 2002; Ranaldi et al., 1996) have been used to study drug partitioning into membranes, mechanisms of drug absorption and drug interaction with epithelial proteins such as transporters and enzymes (Ungell and Karlson 2005).

Other models that use physicochemical methods such as immobilized artificial membrane (IAM) columns (Pidgeon et al., 1995) and parallel artificial membrane permeation assay (PAMPA) (Kansy et al., 1998; Lipinski et al., 1997) have been reported in the literature. These models do not use biological membranes, however, they have been found to be useful in assessing structure-property relationships, such as lipophilicity (Hidalgo et al., 2001), and are advantageous in that they are reproducible and capable of high throughput.

There has been a significant interest in the *in vitro* cell culture techniques because of their simplicity compared with *in vivo* and *in situ* studies (Irvine et al., 1998). The MDCK and Caco-2 cell lines are common models that have been used extensively for studying cell growth regulation, metabolism and transport mechanism.

The Caco2 cell line has been well characterized, is used in the pharmaceutical industry, and is accepted by the regulatory agencies as a drug permeability screening model (Shah

et al., 2006). Caco-2 was derived from the human colon adenocarcinoma cells (Fogh et al., 1977). Under controlled culture conditions on semi-permeable membrane, they proliferate and form a monolayer. The monolayer forms a brush border with well developed microvilli, tight apical junctions and a polarized distribution of membrane components including receptors, transport proteins, ion channels and lipid molecules similar to that found in the absorptive small intestinal epithelial cells (Grasset et al., 1984; Hilgers et al., 1990). They express intestinal enzymes such as amino peptidases, esterases, sulfatases and the cytochrome P450 family of enzymes. Transporters such as bile acid carriers, amino acid carriers, monocaboxylic acid carriers, PEPT1, p-glycoprotein and others have also been reported to be expressed by Caco-2 cells (Dantzig et al., 1990; Hunter et al., 1993; Nicklin et al., 1995; Prueksaritanont, 1996).

Despite their widespread use, concerns have been raised as to inter-laboratory variability in Caco-2 permeability measurements. This has been thought to be caused by what is known as "phenotypic drift" (Hidalgo, 2001), which can be due to different culturing conditions or biological factors associated with the cell culture. As a result it is not encouraged to use permeability data from different laboratories to increase the database to be used in developing *in silico* models; rather this should be limited to a small number of carefully selected compounds (Hidalgo, 2001). Another disadvantage of the Caco-2 cells culture method is low throughput, due in part to limitations regarding its 3 week growth period before it can be used for transport study, regular maintenance and frequent feeding requirements (Irvine et al., 1998).

The MDCK cells have been suggested as an alternative to the Caco-2 cells for permeability measurements. They were originally isolated from dog kidney cortex, and have been shown to retain many of the differentiated properties associated with the kidney tubular epithelium (Herzlinger et al., 1982). When grown on polycarbonate semi-permeable filters, they can generate a trans-epithelial electrical resistance (TEER) demonstrating the presence of functional tight junctions (Herzlinger et al., 1982). Their main advantage over Caco-2 cells is the shorter culture time which can be as short as 2-5 days. Their TEER value is lower than that obtained in Caco-2 cells, and this has been

suggested to be closer to the TEER value of the small intestinal epithelia (Hildago, 2001). As well, permeability values of hydrophilic compounds have been reported to be lower in Caco-2 cells compared with MDCK cells (Kim et al., 1993, Hildago, et al., 1992), suggesting that the tight junctions in MDCK cells is leakier than that in Caco-2 cell cultures.

Other cell types that have been reported in literature include the HT29. Two clones of this cell type have been reported to have the capability to secrete mucus, the HT20-H and HT29-MTX (Hilgendorf et al., 2000). These have been used to study the effects of mucus on the permeability of drugs. However, despite all these attempts using several different techniques, there is no single method that has been proposed as sufficient for studying the drug absorption process, rather a combination of systems are used (Hidalgo, 2001).

1.10 Objectives of the study

The objectives of this study were:

1. To develop dissolution test methods that can be used to forecast *in vivo* drug behaviour.
2. Compare flow-through cell dissolution methods following dynamic pH change protocols with conventional single pH dissolution protocols using the USP Apparatus 2.
3. Utilize the *in vitro* test results as input functions into Gastro plus™ to simulate clinical observed data in order to establish *in vitro/in vivo* correlations.

1.11 Hypothesis

Flow through dissolution test methods following dynamic pH change protocols, combined with *in silico* simulations are able to predict the *in vivo* behaviour of orally administered drug products.

1.12 Significance of the study

The benefits of an *in vitro* tool to predict *in vivo* behaviour to the pharmaceutical industry include:

1. A measure of the likelihood of the success of a planned bioequivalence study.
2. A reduction in the number of bioequivalence studies required for a regulatory submission.
3. Determination of whether *in vivo* performance is limited by the dissolution process or not.
4. Identification of key parameters used for setting dissolution specifications for bioequivalence or API.
5. An ability to provide useful information that can be used to justify an application for bioequivalence studies by biowaivers.

1.13 The drug products studied

Two commercially available drug products, etoricoxib (Arcoxia®) and montelukast sodium (Singulair®) were studied in this project.

1.13.1 Etoricoxib

The first drug product, etoricoxib, also known as [5-chloro-2-(6-methylpyridin-3-yl)-3-(4-methylsulfonylphenyl) pyridine], is a novel orally active agent that selectively inhibits cyclooxygenase-2 (COX-2) (Riendeau et al., 2001). It is a poorly soluble, lipophilic drug with estimated logP of 3.14 and pKa of 4.6. Its pKa predicted using the ADMET predictor™ (version 2.0, Simulations Plus Inc., Lancaster, CA, USA) is 5.18.

Etoricoxib is indicated for the treatment of rheumatoid arthritis, osteoarthritis, acute gout, chronic musculoskeletal pain (including chronic low back pain), postoperative dental pain and primary dysmenorrhoea (Cochrane et al., 2002). The drug is available as oral

tablets and the recommended dosage is 60, 90 or 120 mg/day, with a frequency of administration of once daily.

Etoricoxib behaves like a weak base, due to the presence of the lone pairs of electrons at the nitrogen atoms in the pyridine rings in its structure, which are not delocalized into the aromatic π-system. This makes the pyridine basic with chemical properties similar to that of tertiary amines (Pankratov, 2002). Figure 1-3 show the structure of etoricoxib.

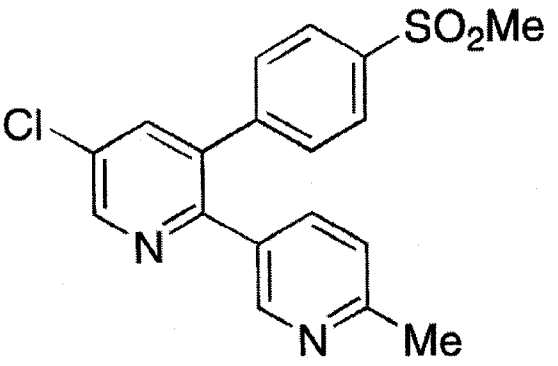

Figure 1-3 Etoricoxib chemical structure

Orally administered etoricoxib is well absorbed with the mean oral bioavailability of approximately 100%. The pharmacokinetics of etoricoxib is linear across the clinical dose range (60 to 120 mg) and T_{max} is reported to be observed at approximately 1 hour after administration to fasted adults (Agrawal, et al., 2003). Etoricoxib is approximately 92% bound to human plasma protein with the volume of distribution at steady state (V_{dss}) of approximately 120 L in humans and half-life of approximately 22 hours. The plasma clearance is estimated to be approximately 50 mL/min (Agrawal, et al., 2003).

1.13.2 Montelukast sodium

The second drug product, montelukast sodium, also known as 1-[[[(1R)-1-[3-(1E)-2-(7-chloro-2-quinolinyl) ethenyl] phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl] propyl]thio]-methyl]-cyclopropane acetic acid, monosodium salt, is a selective and orally active leukotriene receptor antagonist that specifically inhibits cysteinyl leukotriene CysLT1 receptor (LaBelle, 1995; Jones et al., 1995). It is currently used for the treatment

of chronic asthma. It is available as 4 mg granules for paediatric use, 4 mg chewable tablets and 10 mg film coated tablets for adult use.

Montelukast sodium is a highly lipophilic drug with estimated logP of 8.79 and pKa of 2.7 and 5.8 (Thibert et al., 1996). The pKa values predicted using ADMET predictor™ are 5.12 and 9.23 for acid and base, respectively. Its aqueous solubility is reported to be between 0.2-0.5 μg/mL in water at 25°C (Thibert et al., 1996). Because montelukast contains polar and nonpolar groups at opposite ends of the molecule, the drug has amphiphilic physicochemical properties (Thibert et al., 1996). The structure of montelukast sodium is shown in Figure 1-4 below.

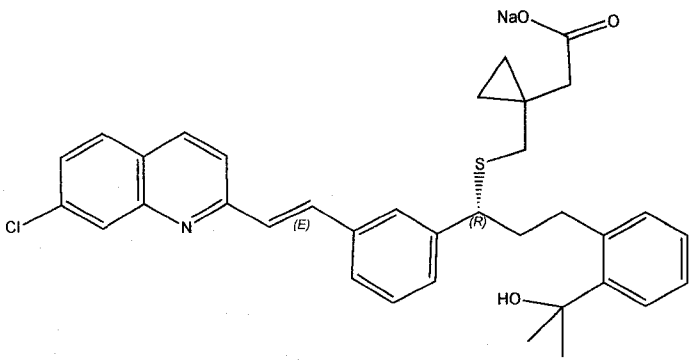

Figure 1-4 Structure of montelukast sodium

Montelukast sodium is reported to be completely absorbed with the mean oral bioavailability (F) of 61% -62% (Zhao et al., 1997). Its plasma clearance is approximately 45.5 ml/min (2.73 L/h)], and a steady-state volume of distribution of approximately 10.5L. The plasma terminal elimination half-life is reported to be 5.1 h with a mean residence time of ~3.9 h (Zhao et al., 1997).

1.14 Format of the dissertation

This dissertation is presented in the traditional format, according to the thesis handbook. Chapter 1 gives a general introduction to the research. Chapter 2 is the materials and methods used in the study. Chapter 3 provides results and discussion on both etoricoxib

(Arcoxia®) and montelukast sodium (Singulair®) and chapter 4 provides some recommendations for future study. An appendix is added to provide the reader an overview of how GastroPlus™ was used throughout this research project.

CHAPTER - 2

2 MATERIALS AND METHODS

2.1 Materials

Etoricoxib API powder (Form-V unmilled Lot # FL00001585), etoricoxib tablets (Arcoxia®-60 mg –lot# L000791456), montelukast sodium API powder (lot # LTA-384) and montelukast sodium tablets (Singulair®-10 mg film coated tablets lot # FL00000908), were provided by Merck Frosst, Canada. Sodium taurocholate (crude bile salts batch # 015K0585), high purity sodium taurocholate (batch # 115K1109, 95% purity), sodium lauryl sulphate (batch # 084K0187), lucifer yellow and trifluoroacetic acid were purchased from Sigma-Aldrich (St Louis, MO). Soy lecithin (phosphatidycholine Lot # 5568H) was purchased from MP Biomedicals Inc, (Solon, Ohio, USA.) and egg phosphatidycholine, Lipoid E PC 99.1% pure (HQ) was purchased from Lipoid GmbH, (Ludwigshafen, Germany). Potassium phosphate monobasic monohydrate, potassium chloride, sodium phosphate monobasic monohydrate, sodium acetate monohydrate, sodium hydroxide, sodium chloride, hydrochloric acid (ACS grade) and glacial acetic acid were purchased from Fisher Scientific (Fisher scientific Canada Inc.). Dichloromethane, methanol and acetonitrile were all HPLC grade. 25 mm, 0.45 micron Whatman glass microfibre filter and 25 mm, 1 micron Acrodisc glass fibre filter were purchased from Life Sciences (Life Sciences Canada Inc).

Dulbecco's modified eagle's medium (DMEM), L-glutamine, trypsin with 0.25% EDTA, HEPES and minimum essential medium (MEM)-non essential amino acids were purchased from GIBCO BRL (Carlsbad, California, USA). Fetal bovine serum (FBS) and Hanks Balanced Salts Solution (HBSS) were purchased from Sigma Aldrich (St. Louis, Missouri, USA). Phosphate buffer saline (PBS) containing 140 mM NaCl, 8.1 mM $Na_2PO_4.H_2O$, 1.47 mM $KH_2PO_4.H_2O$, pH 7.2 was prepared in the lab using chemicals obtained from Sigma (St. Louis, Missouri, USA). Cell culture flasks 75 cm^2, 25 cm^2

growth surface area and Transwell® inserts (24 mm, 0.4 μm pore size, and 4.7 cm^2 growth surface areas) were purchased from Corning Costar (Acton, MA, USA).

2.2 Methods

2.2.1 Media Preparation

Simulated gastric fluid (SGF) pH 1.2 (without enzymes), acetate buffer pH 4, potassium phosphate buffer pH 6.8 and simulated intestinal fluid (SIF) pH 6.8 (without enzymes) were prepared following the USP 27. Simulated gastric fluid (SGF-SLS) pH 2.0, without enzymes but with 0.25% SLS, was prepared as proposed by Dressman et al., (1998).

The biorelevant media containing bile salts and lecithin were prepared following the procedure and modification outlined by Marques (2004), which was adopted from the composition proposed by Galia et al., (1998). The recommended volumes for simulating fasted state conditions (FaSSIF) in the upper small intestine is 500 mL, while for simulating fed state conditions (FeSSIF) in the upper small intestine is 1000 mL (Galia et al., 1998).

2.2.2 Solubility studies in different media

An excess of the drug powder was added into 10 mL of the different media in glass vials. The vials were sealed and placed into a shaking incubator water bath (Dubnoff Metabolic Shaking Incubator- Precision scientific), and the temperature was maintained at 37±0.5° C. Samples were taken at 1, 4, 24 and 48 hrs, filtered using a 0.45 micron Whatman glass microfibre filter (Life Sciences, Canada Inc.) and analyzed by HPLC.

2.2.3 Scanning electron microscopy (SEM) and particle size estimation

Particle size, size distribution estimate and morphology were evaluated using the scanning electron microscope (SEM) (Philips XL, Japan). The instrument was operated at a low vacuum mode at 10 kV. Particle size estimate was performed by measuring the projected area diameter of about 200 particles and using equation 2-1 (Ansel et al., 2005, pp 189), the size distribution was estimated:

$$d_{av} = \frac{\sum nd}{\sum n} \quad \textbf{Equation 2-1}$$

Where: d is the middle value of size range in microns.
n is the number of particles per group.

2.2.4 X-ray powder diffraction pattern (XRPD)

To assess the impact of pressure and dwell time on the powder property, about 60-70 mg API powder were compressed at three different compression pressures and dwell times using a hydraulic lab press (Enerpac P142, Globe Pharma, USA). X-ray diffraction patterns were performed on the compacts using the Scintag XDS-2000 x-ray diffractometer (Scintag Inc. USA). Measurements were taken at a voltage of 45 kV and 40 mA using Si (Li) Peltier-cooled solid state detector. The compression pressure and dwell time that caused the minimum change in powder property was chosen to compress the discs that were used for the intrinsic dissolution tests.

2.2.5 Intrinsic dissolution test

The intrinsic dissolution tests were performed using the static disk intrinsic dissolution apparatus (Distek Inc, New Brunswick, NJ, USA). Compact powder discs of 0.5 cm^2 surface area were prepared by compressing between 60 to 70 mg of the test powder at

2000 PSI for 2 minutes (etoricoxib powder), using a hydraulic lab press (Enerpac P142, Globe Pharma, USA).

The USP apparatus 2 (paddle) and a flat bottom vessel (Distek, New Brunswick, NJ, USA) were used. The distance between the top of the disc and the bottom of the paddle was adjusted to about 2.5 cm, before adding the test media. The water bath temperature was maintained at 37±0.5°C. The test media was added to the vessel and given time to equilibrate (about 45 minutes), and slowly the disc with the compressed powder in it was lowered into the vessel. The test was performed with the spindle operated at 50 RPM, at specified time intervals 5 mL samples were removed from the vessels and replaced with an equal amount of pre-warmed media. Samples were filtered using Whatman glass microfibre filter (25 mm, 0.45 micron) (Life Sciences, Canada Inc.), discarding the first 3 mL. Analysis was done using HPLC (Agilent 1100, USA) equipped with a UV detector and Atlas TS™ data acquisition system.

The intrinsic dissolution rate (IDR) was estimated by dividing the initial slope of the plot of amount dissolved verses time by the surface area of the compact disc exposed to the dissolution media.

2.2.6 Micelle particle size analysis

In order to check for the presence of drug loaded micelles in etoricoxib-SLS solution, saturated solutions of etoricoxib in SGF (without SLS) and in SGF-SLS were subjected to particle size analysis. Mean diameter and polydispersity of the drug loaded micelle in SGF-SLS media were defined by light scattering (3000 HSA Zetasizer Malvern, Zeta-PlusTM zeta potential analyzer, Malvern Instrument Ltd., UK).

2.2.7 Dynamic dissolution tests

2.2.7.1 Equipment set-up and operation

The tests were performed using the custom made flow-through cells (22.6 mm internal diameter, (Scientific Glass Blowing Services, Chemistry Department University of Alberta). Figure 2-1 shows the small cell (11 mm internal diameter) and the large cell (22.6 mm internal diameter). The cells were made following the specifications provided in the USP. (The USP # 27)

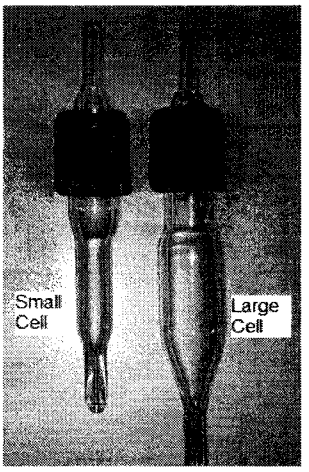

Figure 2-1 The Small and Large flow through cells

A gradient pH change sequence simulating the passage of a drug through the gastro-intestinal tract was used. An open system set-up (Figure 2-2), to enable the test to be performed at the best possible sink condition was used. The media used for the test were placed in a constant temperature water bath, maintained at 37±0.5°C. Two flow-through cells were immersed in the same water bath; a system of rubber tubing running from the media reservoir and passed through a peristaltic pump were used to deliver fresh dissolution media to the cells through the bottom of the cells.

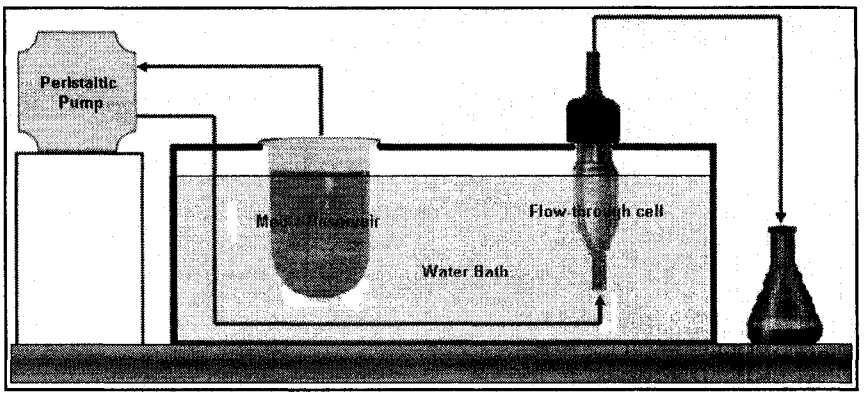

Figure 2-2 Set-up of Flow through cell Dissolution Tester

The filter chambers at the top of the cells were filled with fibre glass wool (~200mg) and the chamber inlet was covered using a wire mesh with fine sieve opening, which was fastened using parafilm. The sieve helps in retaining large un-dissolved particles. The cell tops were secured tightly to prevent air or liquid leakage that may have an impact on the fluid flow. A single 4 mm glass bead was placed at the bottom opening of each cell and one gram of 1 mm glass beads were added on top of the 4 mm glass bead. Placement of the 4 mm glass bead at the bottom helps to prevent blockage of the fluid delivery opening by the 1 mm glass beads.

During the dissolution tests, the entire volume of fluid passing through the cells for each sampling interval was collected in volumetric flasks, weighed and the volume was calculated. Weighing the fluid, rather than using volumetric measurement was found to be much easier, and this also helps to account for the slight differential flow rates in the two cells. A sample was then withdrawn and analyzed by HPLC.

In order to obtain an accurate measurement of the volume of fluid collected, the density of the biorelevant media was previously estimated at room temperature by determining the weight of 100 mL sample (n=3) of the biorelevant medium in 100 mL volumetric flasks. The density of the biorelevant medium was estimated to be 1.004 g/cm^3. This value was used to calculate the volume of fluids collected at each sampling interval during the dissolution tests.

2.2.7.2 Flow through dissolution media change sequence

For etoricoxib, simulated gastric fluid (SGF) consisting of 2 g/L NaCl and 0.25% w/v SLS in 0.01 M HCl, pH 2.0 was pumped through the cells at a flow rate of ~3.3 mL/min for 15 min. This was followed by pumping biorelevant medium (FaSSIF pH 6.5) containing 3.75 mM sodium taurocholate and 0.75 mM lecithin in buffer through the cells for 45 min, at a flow rate of ~5.8 mL/min, followed by FaSSIF pH 7.5 for 90 min and lastly FaSSIF pH 5.0 for 30 min, for a total of 180 min.

For montelukast sodium, the starting medium was simulated gastric fluid (SGF) containing 2 g/L NaCl and 0.1% w/v SLS in 0.01 M HCl, pH 2.0, which was pumped through the cells at a flow rate of ~3.3 mL/min for 15 min. This was followed by pumping biorelevant medium (FaSSIF pH 6.5) containing 3.75 mM sodium taurocholate and 0.75 mM soy lecithin in buffer through the cells for 45 min, at a flow rate of ~5.8 mL/min, followed by FaSSIF pH 7.5 for 150 min and lastly FaSSIF pH 5.0 for 30 min, for a total of 240 min.

2.2.8 Dissolution tests using the USP apparatus 2

Dissolution tests in the USP Apparatus 2 (Erweka DT 6, Germany) were performed using the conventional USP buffers (SIF pH 6.8, phosphate buffer pH 6.8, and simulated gastric fluid without enzymes pH 2.0). For montelukast sodium, an additional test in deionised water with 0.25% SLS was performed. The media volumes used in all the USP apparatus 2 tests were 900 mL. The biorelevant media used was FaSSIF pH 6.5 at the recommended volumes of 500 mL (Galia et al., 1998; Marques, 2004). An additional test in the FaSSIF was performed with 900 mL medium volume. Agitation rate used in all tests was 75 RPM, and 100 RPM was used for montelukast sodium.

At predetermined time intervals, 5 mL samples were taken, and replaced with 5 mL of pre-warmed medium. The samples were filtered using Whatman glass microfibre filter

(25 mm, 0.45 micron) (Life Sciences), the first 3 mL were discarded and the remainder was analyzed by HPLC.

2.2.9 Investigating possible *in vivo* precipitation

To investigate a possible *in vivo* precipitation of etoricoxib under physiological conditions, a transfer model in which a drug solution in simulated gastric fluid is transferred by continuously pumping it into FaSSIF pH 6.5, and a possible drug precipitation is monitored via concentration-time measurements, was used.

An amount of etoricoxib drug powder equivalent to the highest recommended dose (120 mg) was dissolved in 120 mL of simulated gastric fluid (SGF 0.01 M HCl with 2 g/L NaCl) to produce a 1.0 mg/mL solution. The dissolved drug was then pumped using a peristaltic pump (Piper Pump, Dungey Inc. Agincourt, Ontario.), into 500 mL of biorelevant media maintained at 37±0.2°C in a dissolution vessel (Erweka DT 6, Germany) and stirred at 75 RPM using a paddle. The flow rates were ~2.2 and 4.8 mL/min, respectively. The pH in the acceptor vessel was monitored and adjusted using 1N NaOH solution to maintain a pH of 6.5.

5 mL samples were withdrawn at specified time intervals, filtered using a Whatman glass microfibre filter (25 mm, 0.45 micron)(Life Sciences) and analysed by HPLC. The withdrawn amount was replaced with an equivalent amount of pre-warmed media. The concentration of the drug in the total volume of solution was calculated and the theoretical and observed concentrations were plotted vs. time.

2.2.10 Drug permeability assessment using cell culture technique

Drug permeability for etoricoxib was assessed using the Caco-2 cell culture technique. Montelukast sodium permeability was studied using both Caco-2 and the MDCK cell lines.

Caco-2 cells were maintained in growth media, Dulbecco's modified eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 1% non-essential amino acids, 2 mM L-glutamine and HEPES buffer. Cells were cultured in 75 cm^2 flasks, in an incubator maintained at 37° C, an atmosphere of 5% CO_2 and 95% relative humidity. Cells were passaged at 80-90% confluence every 3 to 4 days using trypsin-EDTA solution. Culture inserts (Figure 2-3) were pre-incubated with culture medium for 30 minutes at 37° C and then seeded with 50,000 cells per cm^2 (4.7 cm^2 per insert). The cells were used at passage numbers 50-54 after receipt at passage number 45.

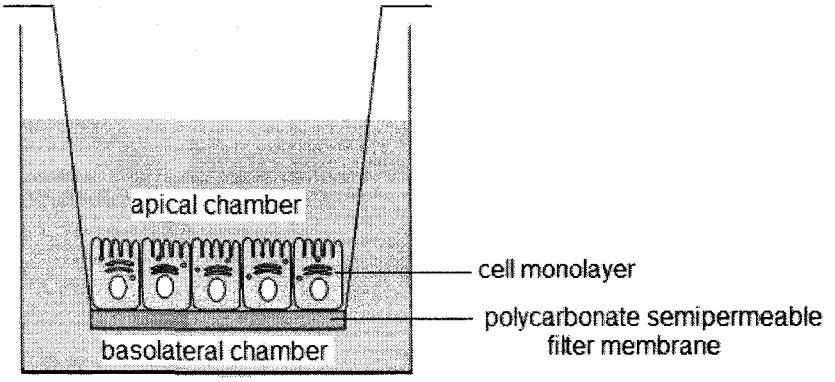

Figure 2-3 Schematic drawing showing cell monolayer on a transwell insert plate

The growth media for MDCK cells consisted of Dulbecco's modified eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 1% non-essential amino acids, 1% sodium pyruvate and 1% glutamic acid. The cells were cultured in 75 cm^2 flasks as well, in an incubator maintained at 37° C, an atmosphere of 5% CO_2 and 95% relative humidity. Cells were passaged at 80-90% confluence every 2 to 3 days using trypsin-

EDTA solution. Culture inserts were pre-incubated with culture medium for 30 minutes at 37° C and then seeded with 40,000 cells per cm^2 (4.7 cm^2 per insert). The cells were used at passage numbers 59-62 after receipt at passage number 55.

2.2.10.1 Cell monolayer screening

Cell monolayers were incubated with the appropriate growth media the day before the assay. On the day of the assay, the monolayers were washed twice with PBS and once with HBSS. The monolayers were equilibrated with the transport medium at room temperature for 30 minutes and the trans-epithelial electrical resistance (TEER) was measured using EndOhm volt-ohm meter (World Precision Instruments, Sarasota, FL, USA). The resistance of the bare filter insert was subtracted from the monolayer resistance values and the results were multiplied by the membrane area of the inserts (4.7 cm^2), to obtain a TEER value for each monolayer ($\Omega.cm^2$). 100 µM of Lucifer yellow was used as a quality control fluorescence marker to verify the integrity of the tight junction, and was measured at 485 nm excitation and 530 emission using a spectrofluorometer (Model: FLUOROMAX, SPEX industries inc., USA).

2.2.10.2 Transport study

The test compound assay consisted of 100 µM etoricoxib solution prepared in HBSS, whose pH was adjusted to 6.5. For montelukast sodium, the test compound consisted of 50 µM solution prepared in HBSS. 1.5 mL of the test drug solution in the transport medium was added to the apical chamber and 3 mL of blank transport medium at pH 7.4 was added to the basolateral chamber for apical to basolateral (A/B) transport assay. For basolateral to apical (B/A) transport, 3 mL of the drug solution (pH adjusted to 7.4) was added to the basolateral chamber and 1.5 mL of blank transport medium was added to the apical surface. The cells were incubated at 37° C, at an atmosphere of 5% CO_2 and 95% relative humidity, and at each sampling interval, the inserts were transferred to new pre-

warmed wells containing blank transport medium. This was done in order to maintain sink conditions in the receiver chamber. The concentration of drug transported was assayed by HPLC and the cumulative receiver concentration was plotted vs. time. After the tests, the TEER in each well was measured again to confirm the integrity of the monolayers.

The effective permeability coefficient (P_{eff}) was calculated using equation 2-2:

$$P_{eff} = \frac{V}{A \times C_0} \times \frac{d_c}{d_t}(cm/s) \qquad \textbf{Equation 2-2}$$

Where dc/dt is the flux across the monolayer (μM/s), and is obtained from the linear slope of the plot of the drug concentration in the acceptor chamber, V is the volume of the receiver chamber (mL or cm^3); C_0 is the initial drug concentration (μM) and A is the surface area of the cell monolayer (cm^2).

2.2.11 Chromatographic conditions

The analytical column used for etoricoxib was Metachem Inertsil-ODS2 10 cm x 3.0 mm, 5μm (Metachem Technolgies Inc.). The column temperature was maintained at 40°C using an external column heater (Eppendoff model TC-50) and the mobile phase consisted of double distilled water with 0.1% trifluoroacetic acid (TFA) and acetonitrile with 0.1% TFA in the ratio H_2O: ACN of 77:23. The mobile phase was filtered using a 0.45 micron nylon membrane filter (Supelco, Supelco Park, Bellefonte, PA, USA). The injection volume was 5 μL and the flow rate was 0.6 mL/min with ultraviolet detection at 280 nm.

The analytical column used for montelukast sodium was Inertsil Phenyl 10 cm x 3.0 mm, 5μm (Metachem Technolgies Inc.), column temperature maintained at 50°C and the mobile phase consisted of double distilled water with 0.2% TFA and acetonitrile with

0.2% TFA in the ratio H_2O:ACN of 50:50. The injection volume was 20 µL and the flow rate was 0.9 mL/min, with detection at 389 nm. The chromatograms were acquired using Clarity™ (version. 2.4.4.83, Data Apex, Prague, Czech Republic) data acquisition software, in cases where Shimadzu LC-600 pump, with SIL-9A auto sampler (Shimadzu, Japan) and Dynamax UV detector (Dynamax Corporation, Elkhart, IN, USA) was used and Atlas TS data acquisition software, in situations where Agilent 1100 series HPLC systems (Agilent, USA) were used.

2.2.12 Computer Simulations using Gastro plus™

Results obtained from the *in vitro* tests were used as input functions into Gastroplus™ version. 5.2.0 (Simulations Plus Inc, Lancaster, CA, USA), to simulate the absorption profiles of the drugs. The three main interfaces (tabs) used for data input are the compound, physiology and pharmacokinetic tabs. Details of how the various data input interfaces in GastroPlus™ were used are shown in Appendix 1. In the compound tab, the basic data pertaining to the physicochemical properties of the drug such as bulk density, solubility, pKa, the dose and particle radius were entered. The human effective permeability for etoricoxib was estimated using Caco-2 data obtained from a study described in the previous section (section 2.2.10.2). GastroPlus™ is equipped with a converter tool that can be used to convert permeability measurement obtained from Caco-2 and MDCK cells lines and from rat intestinal perfusion experiments into human effective permeability. ADMET Predictor™ (version 2.0, Simulations Plus Inc., Lancaster, CA, USA) was used to estimate the human effective permeability for montelukast sodium, as well as the logP value for both drugs. The diffusion coefficients were estimated by Gastroplus™.

The *in vitro* dissolution profiles of the drugs were used as input functions into Gastroplus™ using the tabulated *in vitro* dissolution data input and controlled release-dispersed (CR-dispersed) data input functions. The drug release profiles were used by the software to calculate the drug concentration in each compartment. The estimated human

permeability data were computed using the human LogD absorption model to account for changes in permeability along the GI tract. Gastroplus™ then calculates the fraction dose absorbed based on the ACAT model using drug concentration, permeability, surface area and transit times in each compartment. Values for the pharmacokinetic inter-compartmental rate constants (k_1k_2, k_2k_1 etc), volume of distribution (V_d) and clearance (Cl) were estimated using the PK Plus model in Gastro plus™ and directly imported into the pharmacokinetic tab, to enable the software to calculate the plasma concentration-time curves. In the physiology tab, the default values for the transit times were selected.

The oral plasma (60 mg dose) and the intravenous (IV) (25 mg IV bolus) data used in the simulations and to estimate the PK rate constants for etoricoxib were kindly provided by Merck Frosst Canada. Data for Montelukast sodium were digitized from a published study by Zhao et al (1997), using the program DigIt (version 1.0.2, Simulations Plus Inc., Lancaster, CA, USA), and used to estimate the pharmacokinetic rate constants.
The outputs obtained included the fraction of oral dose absorbed and the plasma concentration-time profile.

2.2.13 Statistical analysis

Similarity (f_2) and Difference (f_1) factors

A simple model independent approach, the pair-wise statistical procedure using the difference factor (f_1) and similarity factor (f_2) (Moore and Flanner, 1996) was used to compare the dissolution profiles obtained from the dissolution tests. The difference factor (f_1) measures the percent error between the two curves and was calculated using the equation 2-3:

$$f_1 = \frac{\sum_{i=1}^{n} |R_i - T_i|}{\sum_{i=1}^{n} R_i} \times 100 \quad \textbf{Equation 2-3}$$

Where n is the number of sampling time points, R_i and T_i are the percent dissolved of the reference and test product at each time points i, respectively. When the test and reference profiles are identical, the percent error is zero, and increases proportionately with an increase in dissimilarity between the two dissolution profiles.

The similarity factor (f_2), which is defined by the food and drug administration (FDA) and the European agency for evaluation of medicinal products (EMEA) as the logarithmic reciprocal square root of transformation one plus the mean squared (the average sums of squares) differences of drug percent dissolved between test and reference, is calculated using the equation 2-4:

$$f_2 = 50 \times \log\left\{\left[1 + \left(1/n\right)\sum_{i=1}^{n}\left|R_i - T_i\right|^2\right]^{-0.5} \times 100\right\} \quad \textbf{Equation 2-4}$$

Where n is the number of sampling time points, R_i and T_i are the percent dissolved of the reference and test product at each sampling time points i, respectively.

Regression analysis

The regression analysis was performed using the built-in functions in Gastro plus™, to establish an *in vitro/in vivo* correlation. Values of the regression coefficient (r^2), the sums of square error of prediction (SSE), the root mean square error (RMSE and the mean absolute error of prediction (MAE) were obtained and compared to observe which of the dissolution profiles best simulates the observed clinical data.

Prediction error statistics

The percent prediction error (PE) was estimated using the equation 2-5 :(Guidance to Industry, FDA 1997):

$$\%PE = \frac{observed - predicted}{observed} \times 100 \quad \textbf{Equation 2-5}$$

Where observed and predicted are either the observed and predicted AUC or the observed and predicted Cmax

2.2.14 Parameter sensitivity analysis (PSA)

Parameter sensitivity analysis was performed using the simulations setup mode in GastroPlus™. A range of values for formulation and compound properties for etoricoxib were selected and a parameter sensitivity analysis was preformed to asses which one of the selected properties has an impact on bioavailability, percent dose absorbed, AUC and C_{max}. The ranges of values used were: Drug particle radius 3 to 300 microns; Drug particle density 0.13 to 13 g/mL; Dose of 20 to 240 mg; Effective permeability 1 to 8 x 10^{-4} cm/s; Reference solubility 0.012 to 1.2 mg/mL.

2.2.15 Estimating first-pass extraction for montelukast sodium

First-pass extraction for montelukast sodium was estimate using the equation 2-6: - (Shargel and Yu, 1999).

$$HepaticER = 1 - \left(\frac{AUC_{oral} / Dose_{oral}}{AUC_{iv} / Dose_{iv}} \right) \qquad \textbf{Equation 2-6}$$

Where HepaticER is the hepatic extraction ratio, AUC_{oral} is the area under the plasma concentration-time curve obtained after administering the oral dose ($Dose_{oral}$) and AUC_{iv} is the area under the plasma concentration-time curve obtained after administering the intravenous dose ($Dose_{iv}$). Briefly, hepatic extraction ratio was calculated using data from a pharmacokinetic study reported by Cheng et al. (1996), then the contributions from non-hepatic extraction was estimated. Based on the drug bioavailability reported, the overall percent of first pass extraction was calculated by adding contributions from hepatic extraction and from non-hepatic extraction. The result was converted to percentage terms and used in the simulations.

CHAPTER -3

3 RESULTS AND DISCUSSIONS

3.1 Etoricoxib results

3.1.1 Solubility studies in different media

The pKa value suggests that the solubility characteristics of the drug would vary considerably *in vivo* as the drug moves along the gastrointestinal tract. The drug equilibrium solubility results (Figure 3-1) indicate that etoricoxib has a high solubility in the gastric media at low pH, and solubility decreases as pH increases. The presence of bile salts and lecithin in biorelevant media does not appear to have an impact on its solubility, thus its solubility in biorelevant media is similar to that in blank buffers.

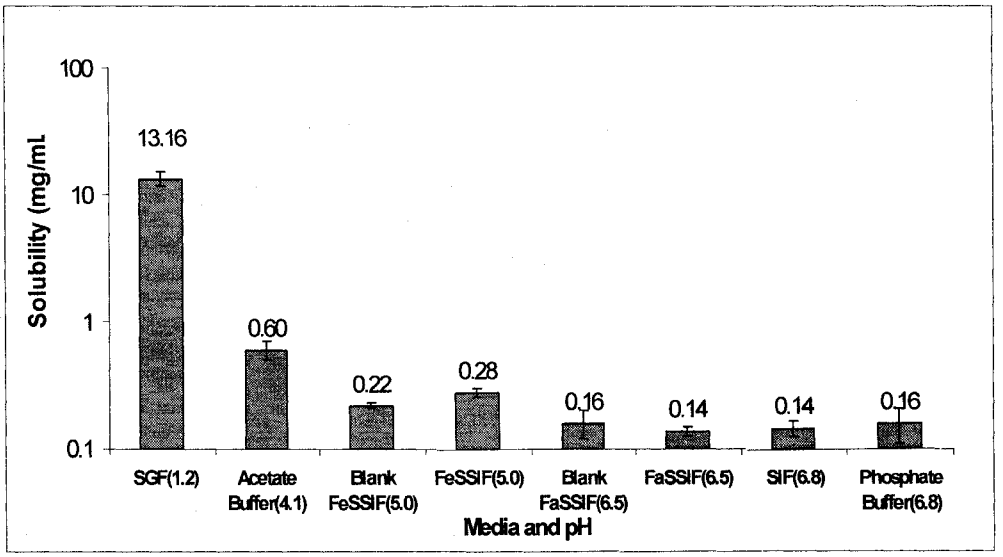

Figure 3-1 Etoricoxib: Solubility in different media

3.1.2 Intrinsic dissolution test

X-ray diffraction patterns obtained by compressing etoricoxib pure drug powder at various compression pressures and dwell times were assessed. This enables the selection of the right compression pressure and dwell time for compressing the drug powder to perform intrinsic dissolution test. Figure 3-2 shows the X-ray diffraction patterns. The

reduction in the sizes of the peaks indicates loss in crystalline structure or conversion to different polymorphic forms (Dalton et al., 2005). Yu et al (2002) suggested that high compression forces might induce polymorphic changes as well, which might result into an incorrect measurement. The compression pressure of 2000 PSI for 2 minutes was chosen to prepare the compacts that were used for the intrinsic dissolution test, since it provided a compact whose x-ray diffraction pattern closely matched that of the raw powder.

Figures 3-3 and 3-4 show the intrinsic dissolution rate profiles of etoricoxib in 4 different media. The results indicate that the intrinsic dissolution rate (IDR), is highest (5.99 mg/min/cm^2) in the USP-SGF without enzymes pH 1.2, followed by (3.06 mg/min/ cm^2) in SGF with 0.25% SLS, pH 2.0. The intrinsic dissolution rates are 0.026 and 0.023 mg/min/ cm^2 in FaSSIF pH 6.5 and FeSSIF pH 5.0, respectively, which compared with the acidic media, are 100 to 200 fold lower.

The presence of drug loaded micelles in saturated solutions of etoricoxib in SGF and in SGF-SLS was assessed by particle size analysis, using a Zetasizer (Model: 3000 HSA, Malven Instruments). It was confirmed that, there were no micelles present in the etoricoxib-SGF solution, but a mean particle size of the drug loaded micelles formed by etoricoxib in SGF-SLS solution was found to be ~672.8 nm (Figure 3-5).

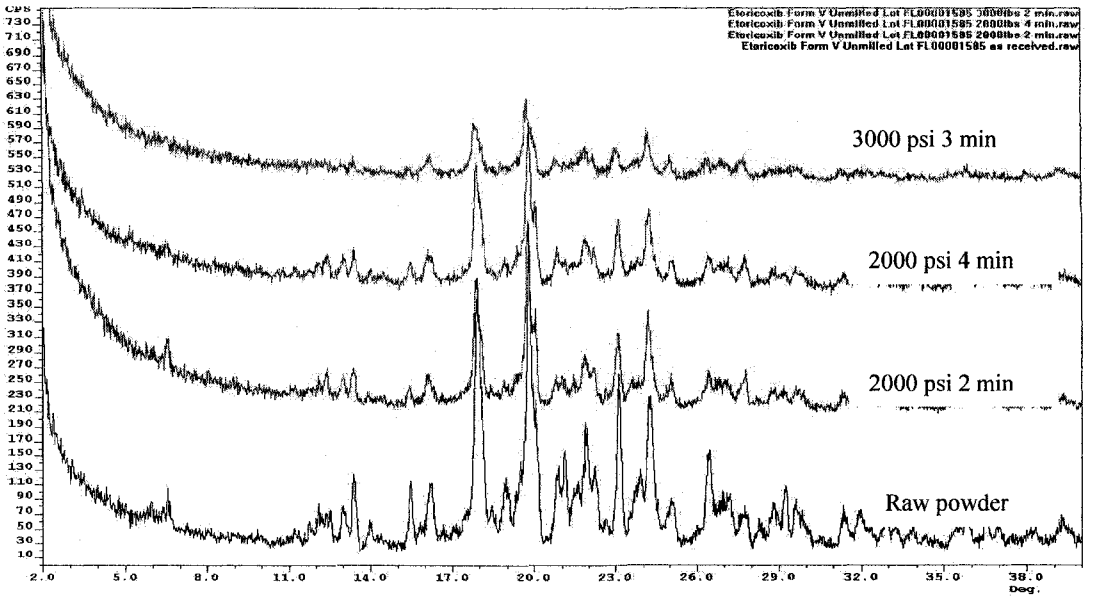

Figure 3-2 Etoricoxib: X-ray diffraction pattern of the API

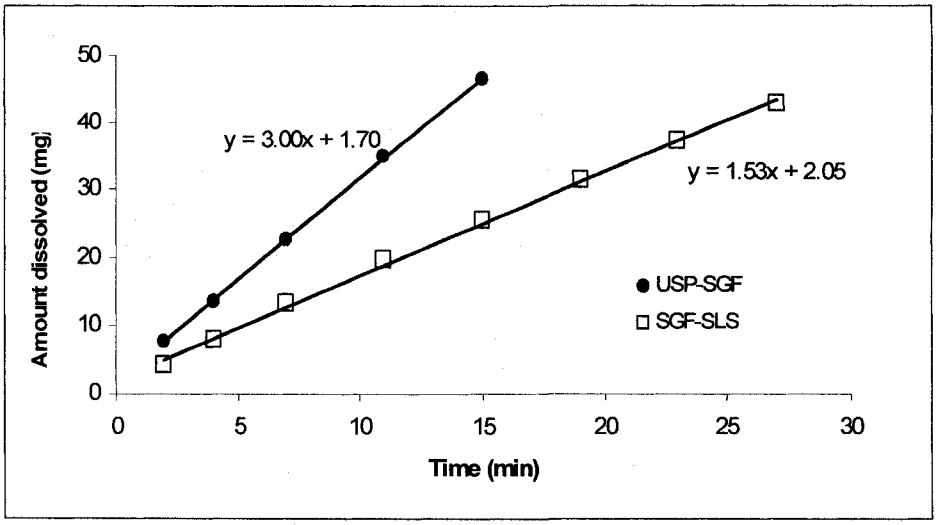

Figure 3-3 Etoricoxib: Intrinsic dissolution profile in USP-SGF and SGF-0.25% SLS

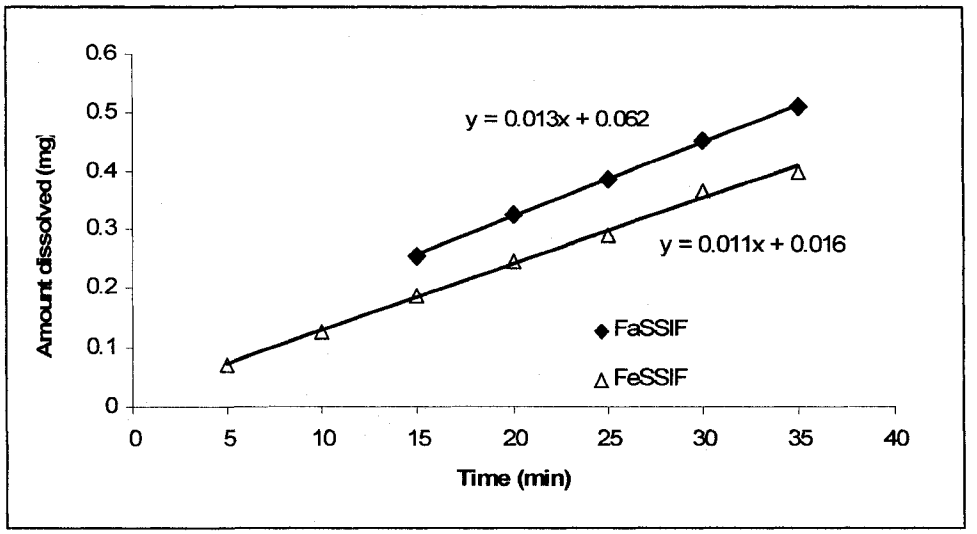

Figure 3-4 Etoricoxib: Intrinsic dissolution profile in FaSSIF and FeSSIF

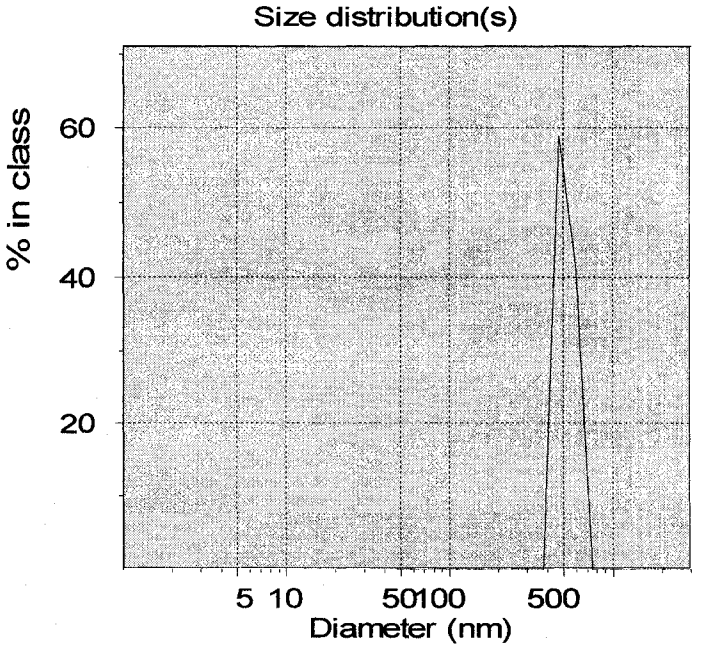

Figure 3-5 Etoricoxib: Particle size distribution of drug loaded micelles formed in SGF-0.25% SLS solution

3.1.3 Particle size evaluation

Figure 3-6 is a scanning electron microscopy (SEM) micrograph of etoricoxib API drug powder. The drug powder can be best described as mainly composed of equant or cubical particles. The particle size distribution can be described as poly-dispersed, approximating a normal distribution with a mean particle diameter of 44.8±26.7 microns (mean ± standard deviation) (Figure 3-7). The particle size distribution (as radius) was used as input into Gastroplus™.

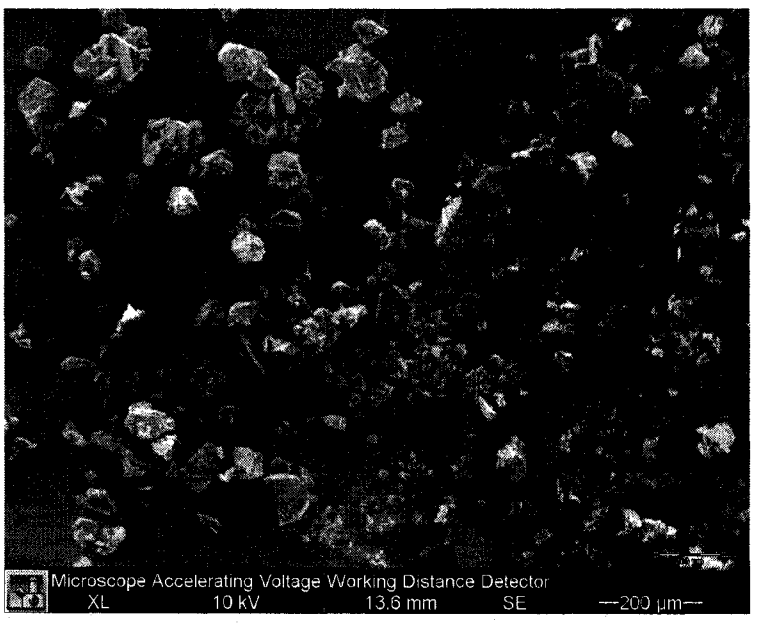

Figure 3-6 Etoricoxib: Scanning electron micrograph (SEM) of the API powder

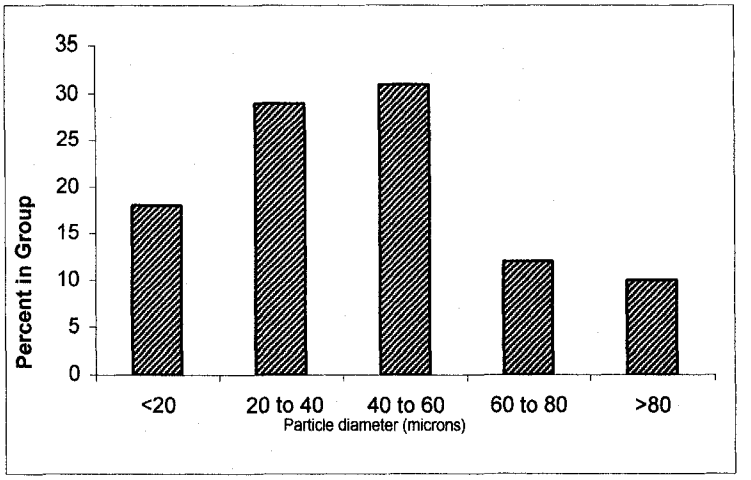

Figure 3-7 Etoricoxib: Particle size distribution of etoricoxib (API) powder measured by projected area diameter of particles counted from SEM scan

3.1.4 Dissolution tests

Figure 3-8 shows the mean dissolution profile of etoricoxib (60 mg tablets) using the flow through cell following the dynamic pH change protocol. The dissolution is fast in SGF at pH 2.0, with greater than 70% dissolved within 15 minutes. The dissolution rate did slow down when the medium was changed to biorelevant medium at pH 6.5 and subsequently to pH 7.5.

Figure 3-9 shows the dissolution profiles of etoricoxib using the USP apparatus 2, as well as the flow through cell for comparison. In all tests, disintegration was fast and complete in less than 5 minutes. The dissolution rate of etoricoxib is fast and undergoes complete dissolution in 5 minutes in 0.01 M hydrochloric acid, pH 2.0, and the rate is slowest in FaSSIF-500 mL compared with FaSSIF-900 mL, phosphate buffer -900 mL and SIF-900 mL. At the end of the dissolution test (90 min), the percentage of the drug dissolved in the different media are:FaSSIF-500 mL, 79.7%; phosphate buffer, 84.3%; the USP-SIF, 84.7%;, FaSSIF-900 mL, 91.6%; and, Flow through cell, 94.3%.

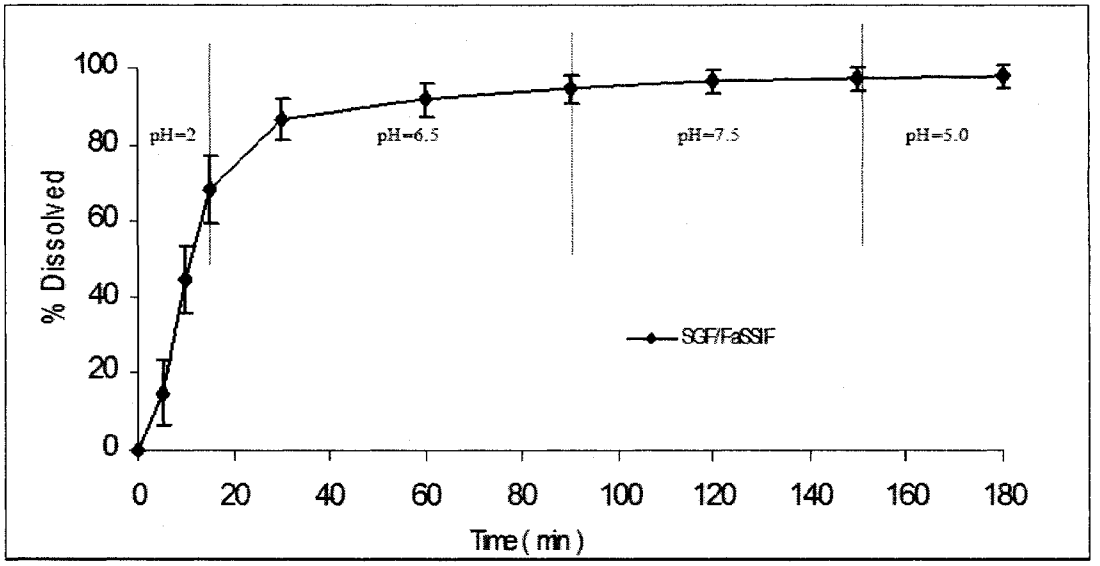

Figure 3-8 Etoricoxib: Dissolution profile in the flow-through cells following the dynamic pH change protocol (n=6)

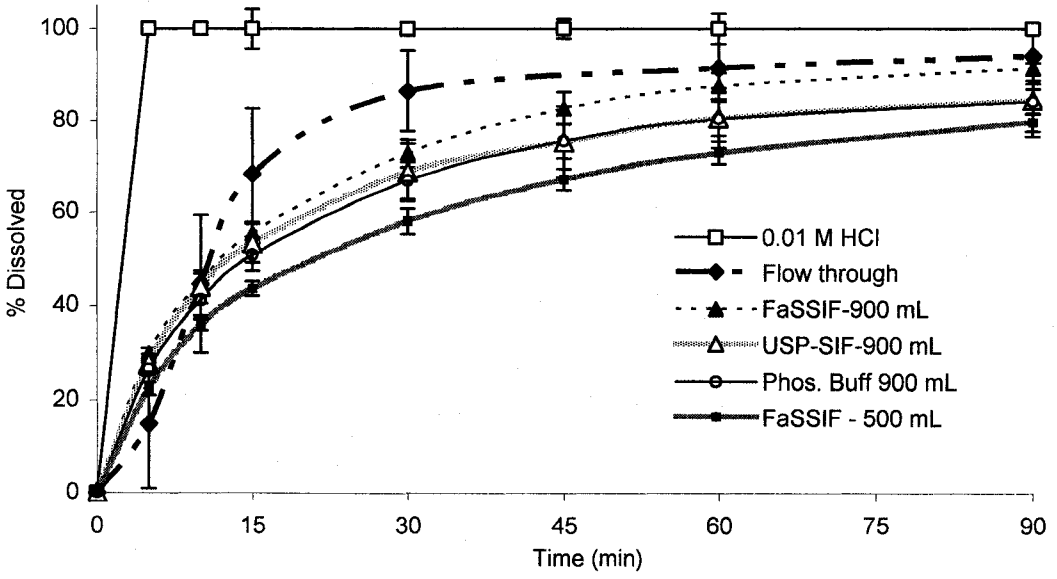

Figure 3-9 Etoricoxib: Comparison of dissolution profiles in the USP Apparatus 2 and the Flow through cell.

(All USP apparatus 2 tests were run at 75 RPM.)

The similarity factor which has been adopted by both the FDA and EMEA, as a criterion for assessment of the similarity between two *in vitro* dissolution profiles was used to compare the dissolution profiles. Both f_1 and f_2 values varies between 0 and 100, and in general, f_1 values lower than 15(0-15) and f_2 values higher than 50 (50 – 100) show that the two dissolution profiles are similar.

Table 3-1 shows the similarity and difference test results, when the profiles from dissolution tests performed in the USP Apparatus 2 are compared, with the profile in biorelevant medium-900 mL as the reference.

Table 3-1 Etoricoxib: Table of f_1 and f_2 test results

	f_1	f_2	Status
FaSSIF-500 mL	10.5	44.8	**Not similar**
USP-SIF	3.3	65.9	**Similar**
Phos. Buff.	4.0	62.3	**similar**
The profile in the FaSSIF-900 mL **was used as reference**			

3.1.5 Investigating possible in vivo precipitation under physiological conditions

Figure 3-10 shows the mean concentration-time profile of etoricoxib when dissolved in simulated gastric fluid and added into 500 mL of SIF and FaSSIF at 2.2 mL/min, and into FaSSIF at flow rate of 4.8 mL/min. The final concentration of the drug appears to be higher than the solubility of the drug predicted in FaSSIF, or in SIF with no precipitation observed within two hours.

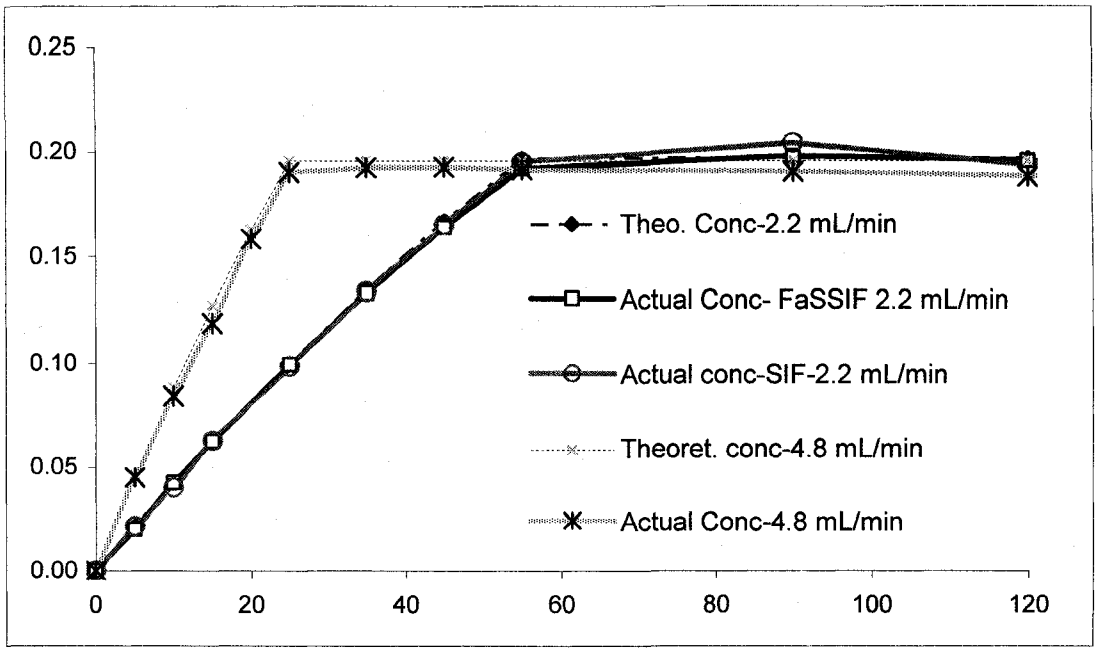

Figure 3-10 Etoricoxib: Measured and theoretical concentrations when a 1 mg/mL solution in SGF was added to FaSSIF.

3.1.6 Cell Culture permeability studies

The Caco-2 cell culture permeability results showed that the apical/basolateral (A/B) transport was 5.23 $x10^{-5}$cm/s and the basolateral/apical (B/A) transport was 5.07 $x10^{-5}$ cm/s. The permeability directional ratio (PDR), which is the ratio of BA/AB, was estimated to be 0.969. The human jejunum effective permeability (P_{eff}) was estimated using the permeability converter utility in Gastroplus™, using the *in vitro* Caco-2 permeability data. The value obtained was 4.07 x 10^{-4} cm/sec. The human effective permeability estimated using ADMET Predictor™ version. 2.0 (Simulations Plus Inc, Lancaster, CA, USA), was 3.5 x 10^{-4} cm/sec.

3.1.7 Computer simulations

3.1.7.1 Simulations using dissolution data

Figure 3-11 shows simulated plasma profiles using dissolution data from the flow through cell dynamic dissolution protocol, and from the USP apparatus-2, 0.01 M HCl, FaSSIF pH 6.5- 500 mL and 900 mL, the USP-phosphate buffer pH 6.8, the USP-SIF pH 6.8. All the six simulated profiles appear to be similar; however there are differences in the C_{max} (maximum plasma concentration) obtained. The simulated C_{max} is lower in the profiles simulated using the dissolution profile from the USP-SIF, the USP-phosphate buffer and FaSSIF-500 mL, compared with profiles from 0.01 M HCL, the flow through cell and FaSSIF-900 mL. The compartmental absorption patterns (Figure 3-12) shows that all the dissolution profiles predict nearly 100% of the drug absorbed, however in the flow through cell, 0.01 M HCl, and FaSSIF 900 mL, 79.7, 78.7 and 71.9% of the drug is absorbed from the upper GI tract, and less than 10% is absorbed from the caecum and the colon combined. While in SIF, phosphate buffer and FaSSIF-500 mL, only 66.7, 66.2 and 62.1% of the drug is absorbed from the upper parts of the GI tract and greater than 10% is absorbed from the caecum and colon combined.

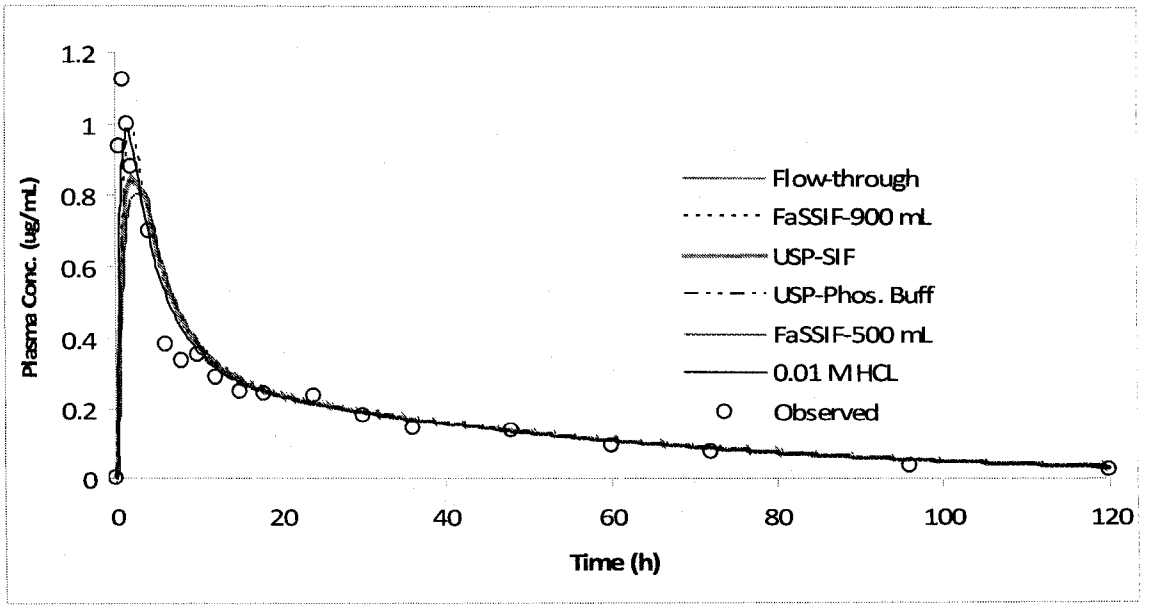

Figure 3-11 Etoricoxib: Comparison of simulated profiles using dissolution data

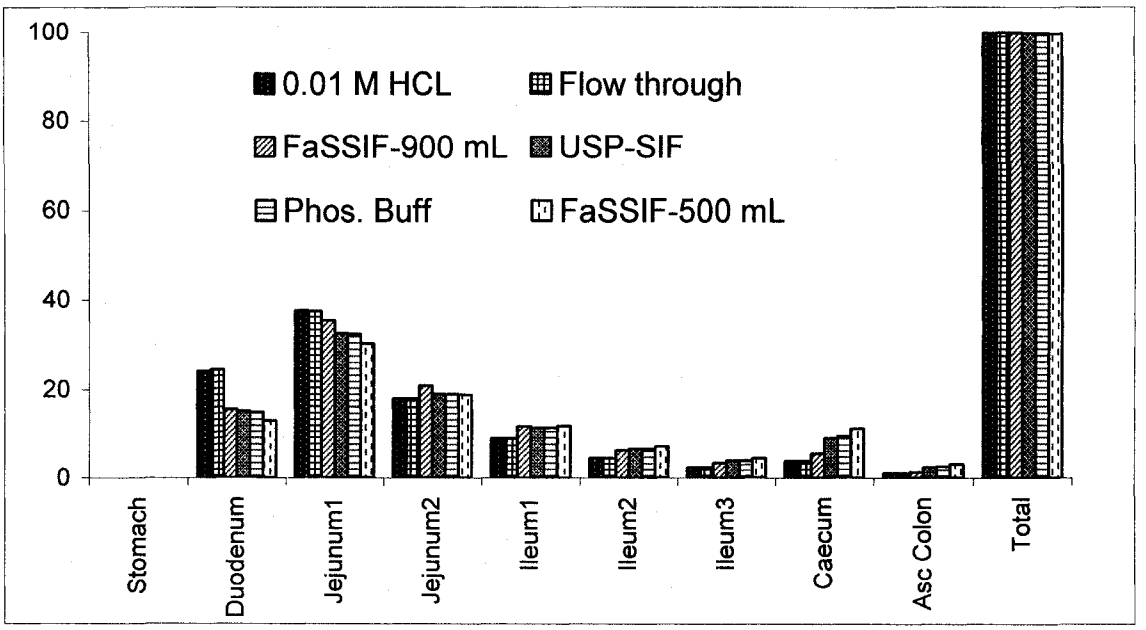

Figure 3-12 Etoricoxib: Compartmental absorption patterns from simulation results in figure 9

3.1.7.2 Simulations as oral solution

A set of simulations were performed to investigate if etoricoxib absorption profile is similar to an oral solution. First a hypothetical dissolution profile in which 100% of the drug is dissolved in 1 minute was created and simulated using the controlled release (CR) disperse system. Then a simulation using the oral solution model was performed. The simulated profiles are shown in Figure 3-13. A comparison of the simulated profiles with that simulated using the flow through dissolution data shows that all the profiles are similar and superimposable. The compartmental absorption patterns (Figure 3-14) show that 79.5%, 79.7% and 78.4% of the drug is absorbed from the upper part of the gastrointestinal tract, using the flow through, CR-1 minute dissolution profiles and solution model respectively, with less than 10% absorbed from the caecum and colon combined.

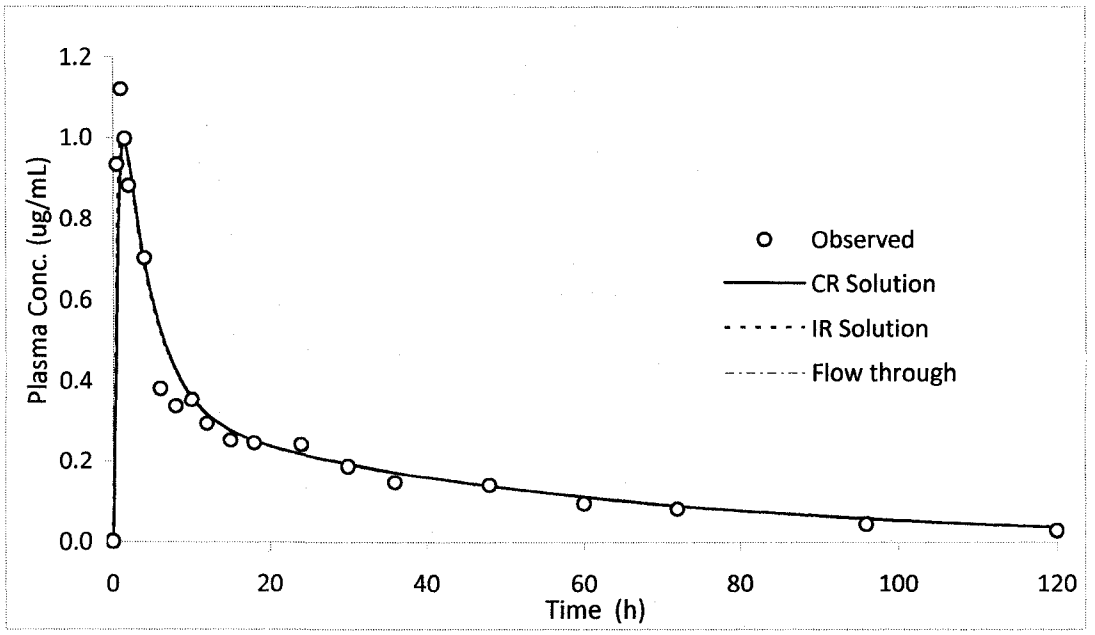

Figure 3-13 Etoricoxib: Comparison of simulated profiles

Note: The above comparison was done using profiles simulated as a controlled release solution (CR) an immediate release solution (IR), and dissolution data from Flow through cell

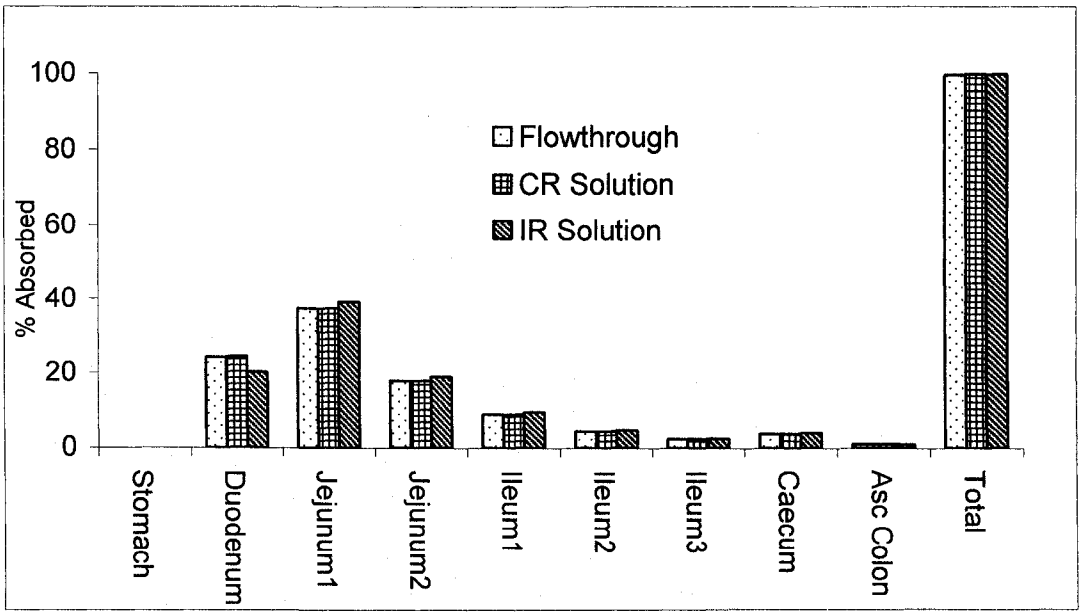

Figure 3-14 Etoricoxib: Comparison compartmental absorption patterns

Note: The above comparison was done using profiles simulated as a controlled release solution (CR) an immediate release solution (IR), and dissolution data from Flow through cell

3.1.8 Statistical analysis

A regression analysis results to compare the simulated and observed profiles is shown in Table 3-2. The results indicate that better *in vitro/in vivo* correlation can be established using dissolution data from the flow through dissolution cell, when simulated as solution, the FaSSIF-900 mL and in 0.01 M HCl in that order (r^2 = 0.90, 0.90, 0.899, 0.898, respectively). Dissolution profiles from SIF, Phosphate buffer and FaSSIF 500 mL provide weak correlations with the *in vivo* data (r^2 = 0.676, 0.668 and 0.593, respectively). The percent prediction error (PE) shown in Table 3-3 indicate that the flow through cell, the FaSSIF-900 mL and the 0.01 M HCl dissolution data predict both the AUC and Cmax as well as when simulated as solutions, and better than the USP-SIF, Phosphate buffer and FaSSIF 500 mL.

Table 3-2 Etoricoxib: Regression analysis output

	Power of prediction Values			
Medium/Method	**r^2**	**SSE**	**RMSE**	**MAE**
Flow through cells	0.900	0.195	0.101	0.054
CR-Solution	0.900	0.193	0.101	0.054
FaSSIF-900 mL	0.899	0.195	0.101	0.058
0.01 M HCL	0.898	0.197	0.102	0.054
USP-SIF	0.676	0.613	0.180	0.093
Phosphate Buffer	0.668	0.634	0.183	0.095
FaSSIF-500 mL	0.593	0.820	0.208	0.114

Table 3-3 Etoricoxib: Percent prediction error (PE) statistics

Observed values: AUC=1.818 x 10^4 ng.h/mL C_{max}=1.12 µg/mL				
			%PE	%PE
Media	**AUC (ng.h/mL)**	**C_{max} (ug/mL)**	**AUC**	**C_{max}**
0.01 M HCL	1.942 x 10^4	0.989	-6.82	11.69
Flow through cells	1.942 x 10^4	0.989	-6.82	11.74
FaSSIF-900 mL	1.941 x 10^4	0.990	-6.77	11.59
Phosphate Buffer	1.938 x 10^4	0.839	-6.60	25.05
USP-SIF	1.939 x 10^4	0.844	-6.66	24.64
FaSSIF-500 mL	1.937 x 10^4	0.806	-6.55	28.04
CR-Solution	**1.942 x 10^4**	**0.989**	**-6.82**	**11.74**
IR Solution	**1.943 x 10^4**	**1.007**	**-6.88**	**10.05**

-ve sign means predicted is > mean observed, and no sign means predicted is < observed.

3.1.9 Parameter sensitivity analysis (PSA)

The outputs from parameter sensitivity analysis indicate that within the range of values tested, both bioavailability and percent dose absorbed are not sensitive to any of those parameters. AUC and C_{max} were found to be sensitive to the dose, in that they will increase with increasing dose, and C_{max} is very slightly sensitive to effective permeability (Figure 3-15).

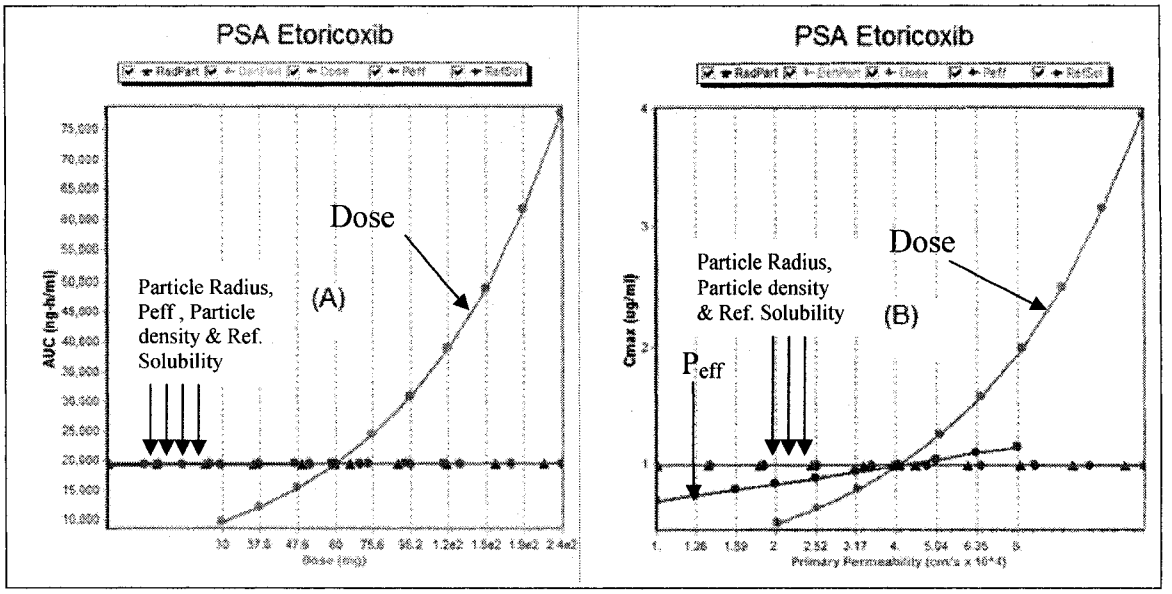

Figure 3-15 Etoricoxib parameter sensitivity analysis output

Comparison of the effect of varying particle radius, particle density, dose, effective permeability and reference solubility on AUC and C_{max}

In figure 3-15(A), the horizontal data points and lines show the effect of increasing particle radius, particle density, dose, effective permeability and reference solubility on AUC. The horizontal parallel lines indicate that within the range of values tested, Particle radius, Particle density, Effective permeability and reference solubility have no effect on AUC; however, dose has an effect on AUC and AUC will increase as the dose increases.

In figure 3-15(B), the horizontal data points and lines show the effect of particle radius, particle density, dose, effective permeability and reference solubility on C_{max}.
The horizontal parallel lines indicate that within the range of values tested, particle radius, particle density, and reference solubility have no effect on C_{max}; however, dose has an effect on C_{max} and C_{max} will increase as the dose increases. Effective permeability also has an impact on C_{max}, indicating that C_{max} would only change slightly if effective permeability was varied within the range of values tested.

3.2 Montelukast sodium results

3.2.1 Solubility in different media

Results from solubility studies indicate that montelukast sodium has a low solubility at low pH in the USP-simulated gastric fluid pH 1.2, 0.00018 mg/mL, but in the presence of sodium lauryl sulphate at pH 2.0, its solubility increases by more than 1000 fold to 0.24 mg/mL. The solubility of montelukast sodium increases 5 fold between pH 4.0 and 5.0 and there is no significant difference in solubility in the pH range of 5 to 7.5 in blank buffers (Figure 3-16). Its solubility in biorelevant media is higher than in the corresponding blank buffers at the same pH. The highest solubility was observed in biorelevant media at pH 7.5, and is even higher in media prepared using high quality bile salts (4.69 mg/mL) compared with media prepared using crude bile salts (3.37 mg/mL) (Figure 3-17).

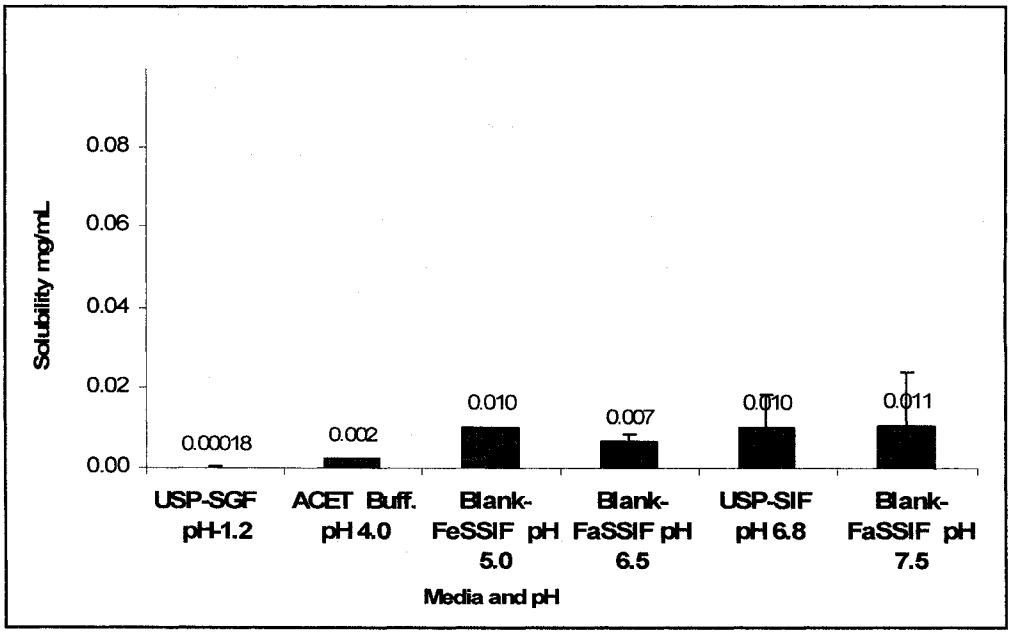

Figure 3-16 Montelukast sodium: solubility in blank biorelevant media and USP-Buffers

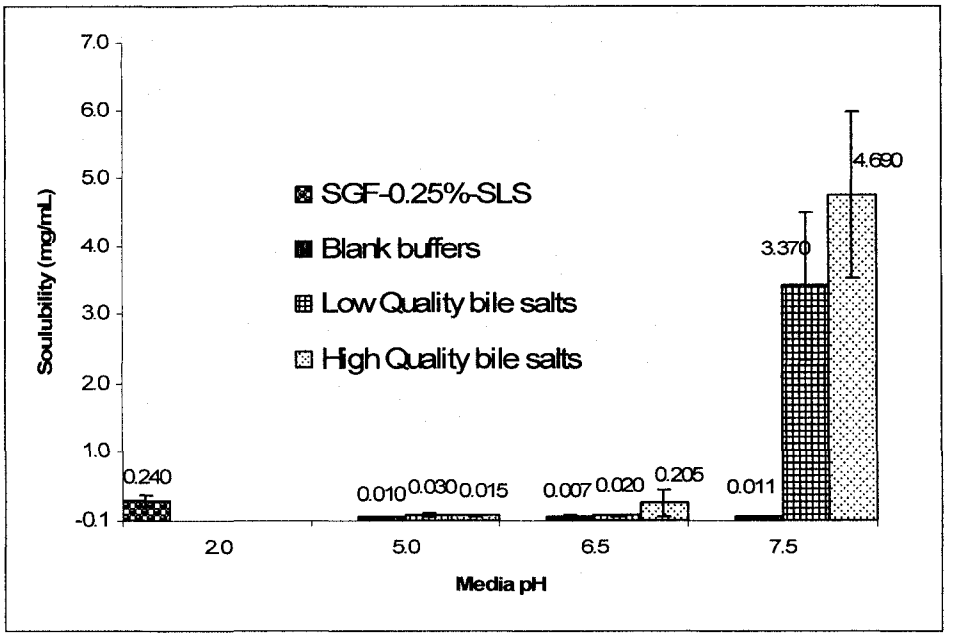

Figure 3-17 Montelukast sodium: solubility in SGF with 0.25% SLS, blank and biorelevant media containing High and Low quality bile salts and lecithin.

3.2.2 Intrinsic dissolution test

Figure 3-18 shows the X-ray diffraction patterns of montelukast sodium powder compressed at various compression pressures and dwell times. There was a significant loss in crystallinity as the compression pressure or the dwell time was increased. As a result of the observed loss in crystallinity, intrinsic dissolution test was not performed for montelukast sodium. To perform the test under such conditions would not reflect the true intrinsic dissolution rate of the drug. A high compression force that causes loss of crystallinity might induce polymorphic changes, which might result into an incorrect measurement of the intrinsic dissolution rate (Yu et al., 2002).

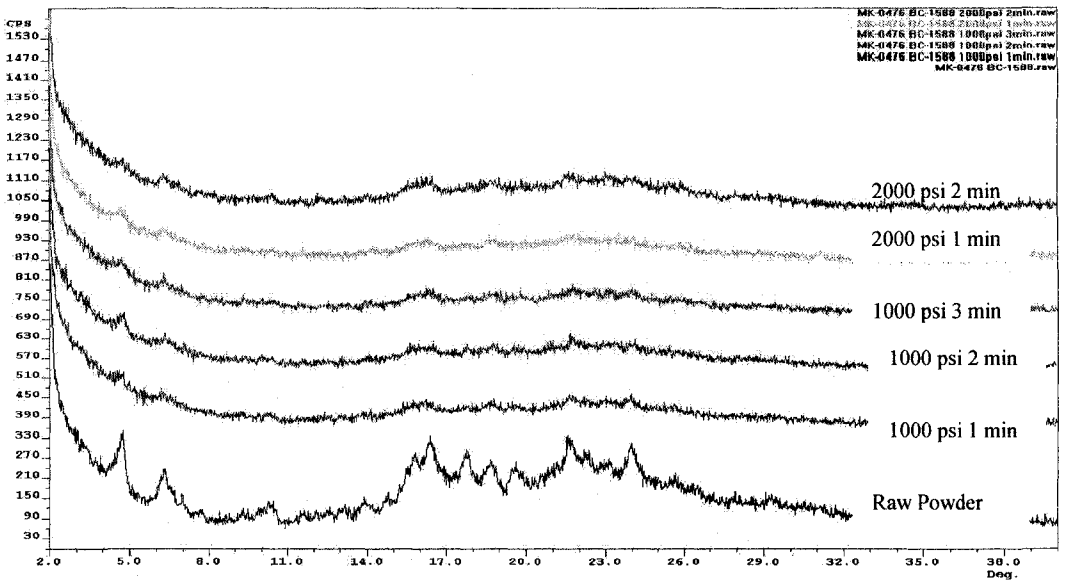

Figure 3-18 Montelukast sodium: X-ray powder diffraction pattern

3.2.3 Particle size evaluation

Figure 3-19 shows a SEM micrograph of montelukast sodium powder. The drug powder can be best described as mainly composed of variously shaped particles, mainly needle shaped and flakes. The particle size distribution can be described as poly-disperse, with small particle sizes. The mean particle diameter was estimated using the microscopic method to be 11.56±9.7 microns (mean ± standard deviation) (Figure 3-20). The particle size distribution (as radius) was used in Gastroplus™ and used as an input support file during simulations.

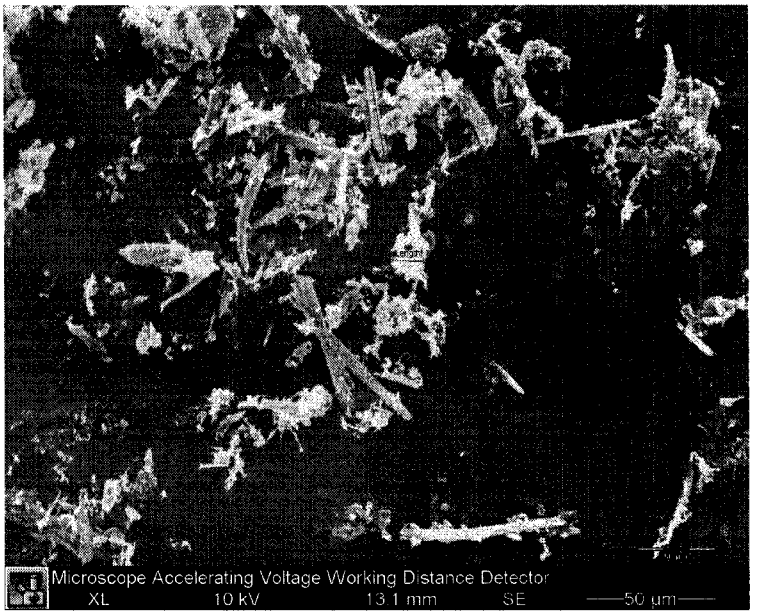

Figure 3-19 Montelukast sodium: SEM micrograph of the API powder

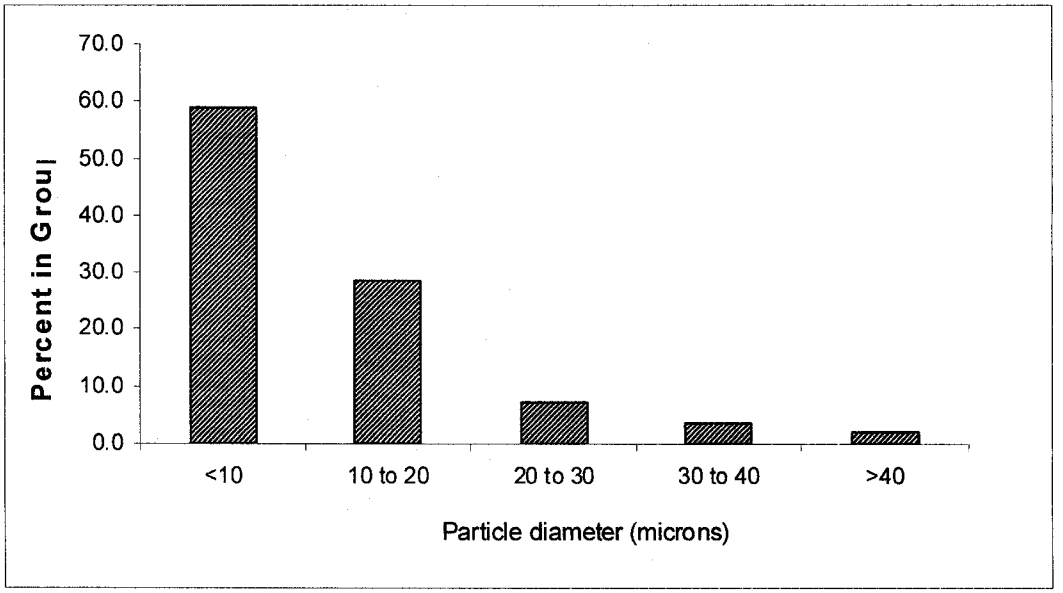

Figure 3-20 Montelukast sodium: particle size distribution

3.2.4 Dissolution tests

Figure 3-21 shows the mean dissolution profile of montelukast sodium (10 mg tablets) using the dynamic flow through protocol. The dissolution rate changes as the pH of the medium is changed, with a fast dissolution rate observed in SGF-0.1% SLS at pH 2.0. The dissolution rate slows down in biorelevant medium at pH 6.5, and then increases when the medium was change to that at pH 7.5.

The mean dissolution profiles of montelukast sodium (10 mg tablets) performed in the USP apparatus 2 using various media is shown in Figure 3-22. Dissolution is fast and complete in 10 minutes when performed in water with 0.25% SLS. This is a significant difference compared with the dissolution in biorelevant media.

Better dissolution was observed in biorelevant media compared with the USP buffers and blank biorelevant media, with similar pH values. At the end of the dissolution test (240 minutes), 88.9%, 76.7% and 69.4% of the drug was dissolved in FaSSIF-500 mL-100 RPM, FaSSIF-900 mL-75 RPM and FaSSIF-500 mL-75 RPM, respectively. In the USP-SIF and in phosphate buffer, less than 10% of the drug is dissolved, even when the test was performed for up to 240 minutes, while dissolution in blank FaSSIF pH 6.5 was practically non existent.

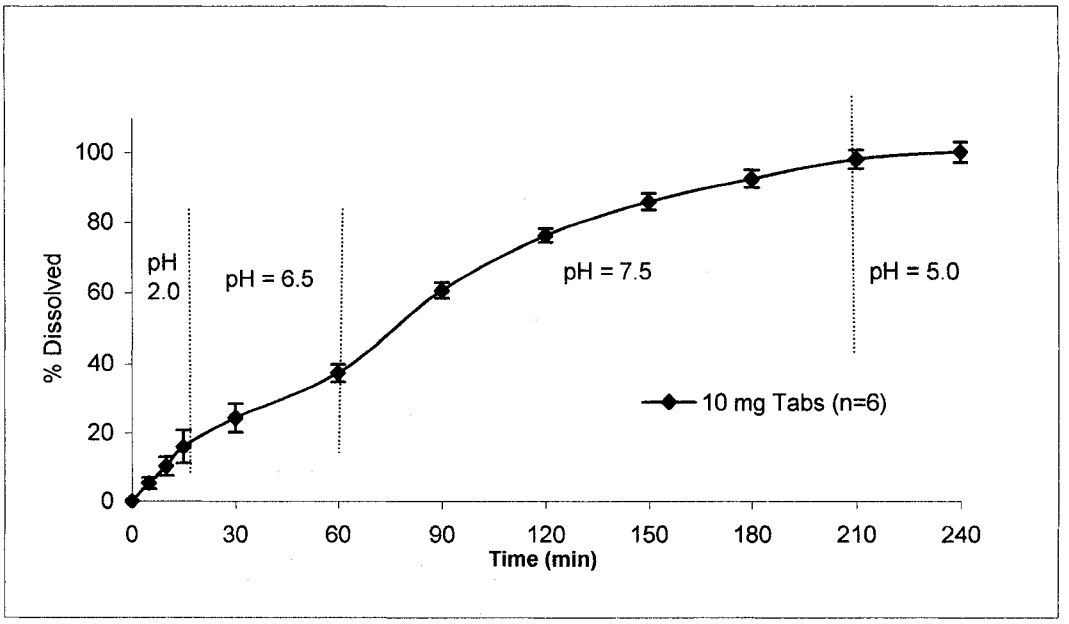

Figure 3-21 Montelukast sodium: Dissolution profile in the flow-through cells following the dynamic pH change protocol (n=6)

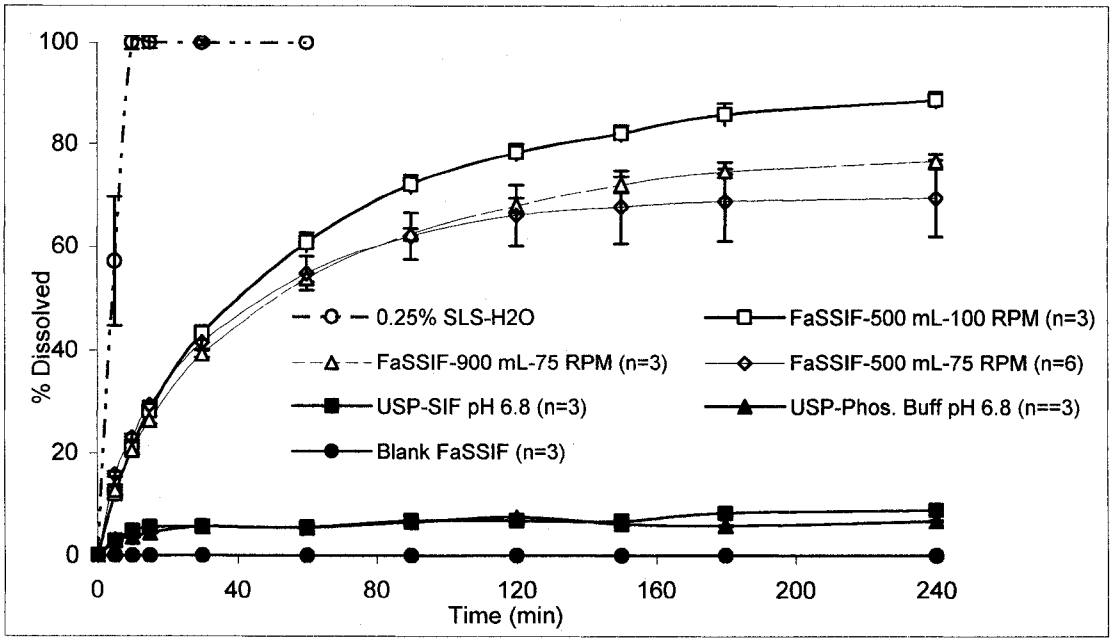

Figure 3-22 Montelukast sodium: Dissolution profiles in various media using the USP apparatus-2

Table 3-4 shows the similarity and difference factor test results, when the profiles from dissolution tests performed in the USP Apparatus 2 are compared, with the profile in biorelevant medium-500 mL-100RPM in the same apparatus is used as reference.

Table 3-4 Montelukast sodium: table of f_1 and f_2 test results

	f_1	f_2	Status
FaSSIF-900 mL75 RPM	5.0	54.6	**Similar**
FaSSIF-500 mL75 RPM	6.9	48.0	**Not similar**
Phos. Buff.	333.7	11.8	**Not similar**
USP-SIF	292.5	12.1	**Not similar**

*The profile in the **FaSSIF-500 mL-100 RPM** was used as reference*

3.2.5 Estimating first pass extraction

First-pass hepatic extraction ratio calculated using data from a pharmacokinetic study reported by Cheng et al., (1996), was 0.33. This means that 33% of the drug absorbed is lost through hepatic first pass metabolism. With a reported mean bioavailability of 62%, if 100% of the drug is absorbed, this means that the total loss through first pass extraction should be about 38%. If 33% is lost through liver metabolism, it means that 5% is extracted through non-hepatic route. This is most likely through contribution from the gut wall metabolism. A 38 % first pass extraction was then used in the simulations.

3.2.6 Computer simulations using dissolution data

Figure 3-23 show the simulated profile using the flow through cell dissolution data, with and without first pass extraction correction. Figure 3-24 shows the observed and simulated profiles using the flow through cell dissolution data and from the USP Apparatus 2, in different media type, volume and with different paddle speed. In all simulations, first pass extraction correction was applied. The simulated profile from the

surfactant solution (0.25% SLS in water), however, over-predicted the C_{max}, while the in the USP-SIF and phosphate buffer (where less than 10% of the drug is dissolved), resulted in profiles close to baseline. Simulations using the flow through dissolution data appear to match the *in vivo* profile well, compared with simulations using dissolution data from biorelevant medium in the USP Apparatus 2.

Linear regression results are shown in Table 3-5 and the prediction error (PE) statistics are shown in Table 3-6. Simulations using dissolution data from the flow through cell provides the best IVIVC ($r^2 = 0.979$), followed by simulations using data from FaSSIF-500 mL at 100 RPM in the USP Apparatus 2 ($r^2 = 0.834$). The simulations using dissolution profile from the USP Apparatus 2 using FaSSIF-900 mL-75 RPM, FaSSIF-500 mL-75 RPM and in water with 0.25% SLS, result into weak correlations, with $r^2 =$ 0.756, 0.683 and 0.58 , respectively. The USP-SIF and phosphate buffer have almost no correlation at all. The SSE, RMSE and MAE also increase as the correlation gets weaker. The prediction error statistics show that the only profiles that predict the *in vivo* profile of montelukast sodium better are those in the flow through cell and FaSSIF-500 mL-100 RPM.

The compartmental absorption pattern shown in Figure 3-25, indicates that montelukast sodium absorption takes place throughout the GI tract. The absorption pattern from the surfactant solution (H2O-0.25% SLS), however, shows that 90 % of the drug is absorbed from the upper parts of the GI tract. The pattern from the flow through cell shows that about 56% is absorbed from the upper parts of the GI tract, while between 50% and 60% is as absorbed from the same upper parts, from profiles simulated using dissolution data in biorelevant medium using the USP Apparatus 2.

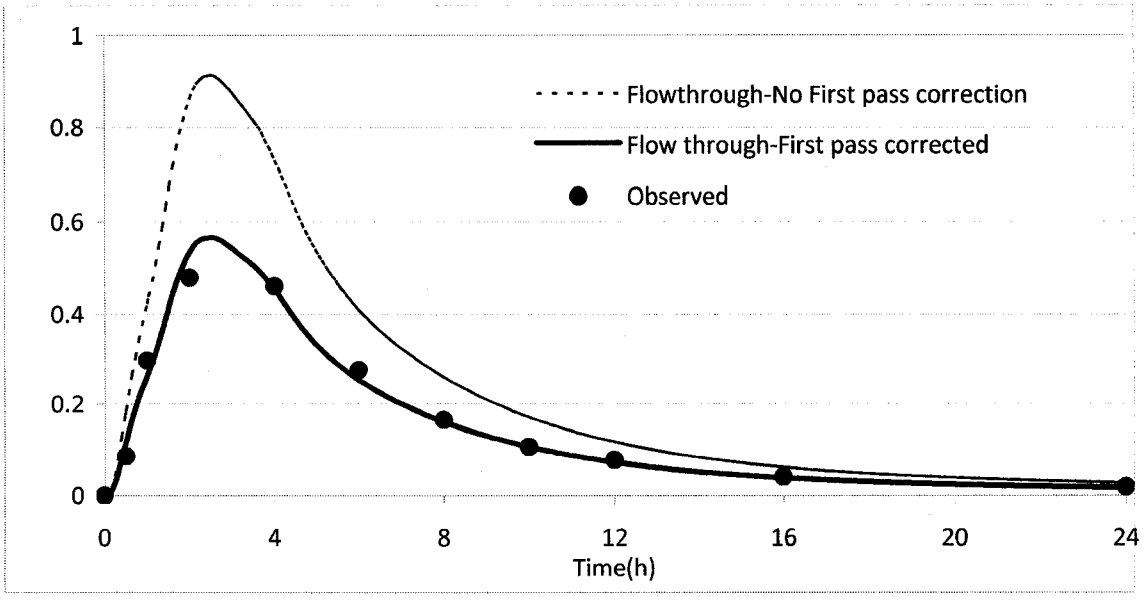

Figure 3-23 Comparison of simulated profiles from the flow through without first-pass extraction correction (No FPE), with profiles where first-pass metabolism was applied

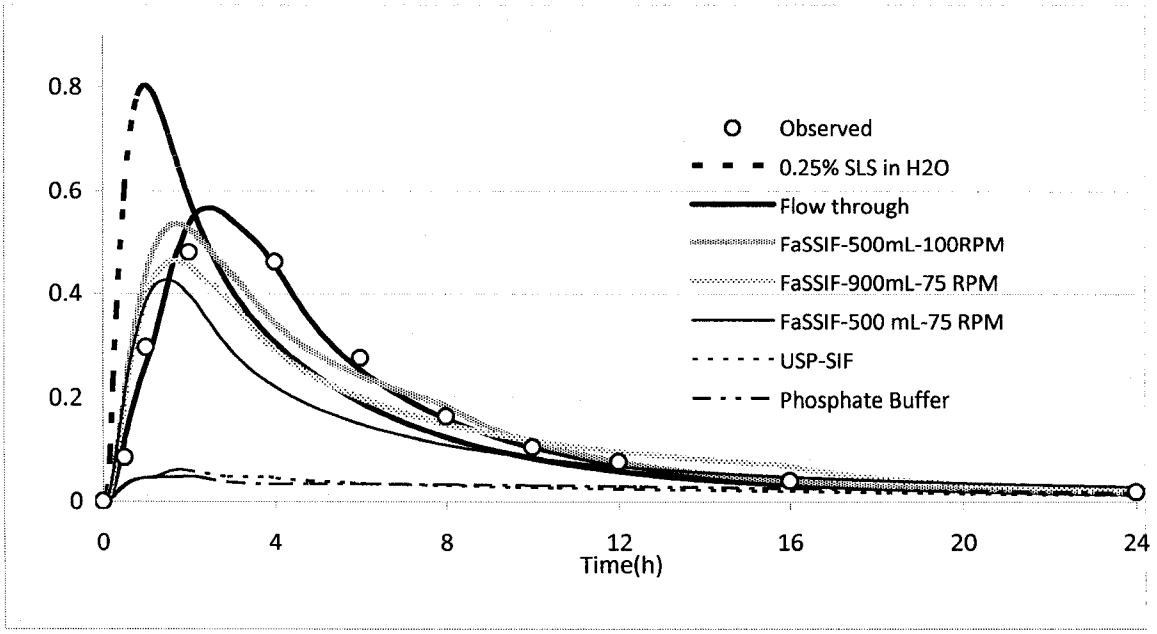

Figure 3-24 Montelukast sodium: Comparison of simulated profiles from different dissolution media

Table 3-5 Montelukast sodium: Linear regression analysis

	Power of prediction Values			
Medium/Method	**r^2**	**SSE**	**RMSE**	**MAE**
Flow through cells	0.979	0.006	0.024	0.016
FaSSIF-500 mL-100 RPM	0.834	0.053	0.078	0.049
FaSSIF-900 mL-75 RPM	0.756	0.057	0.076	0.055
FaSSIF-500 mL-75 RPM	0.683	0.093	0.096	0.071
0.25% SLS-H_2O	0.580	0.567	0.238	0.146
USP-SIF	0.359	0.526	0.229	0.171
Phosphate Buffer	0.354	0.517	0.227	0.168

Table 3-6 Montelukast sodium: Percent prediction error (PE) statistics

Observed values	**AUC=3.552 µg.h/mL**	**C_{max}=0.4796 µg/mL**		
Media	**AUC (ug.h/mL)**	**C_{max} (ug/mL)**	**AUC %PE**	**C_{max} %PE**
0.25% SLS H2O	3.54	0.803	0.4	-67.4
Flow through cells	3.52	0.567	1.0	-18.2
FaSSIF-500 mL-100 RPM	3.49	0.535	1.7	-11.6
FaSSIF-900 mL-75 RPM	3.31	0.462	6.9	3.6
FaSSIF-500 mL-75 RPM	2.68	0.426	24.5	11.2
USP-SIF	0.64	0.061	81.9	87.3
Phosphate Buffer	0.68	0.048	80.9	90.0
Flow thru-No FPE	5.67	0.913	-59.6	-90.3

-ve sign means predicted is > mean observed, and no sign means predicted is < observed.

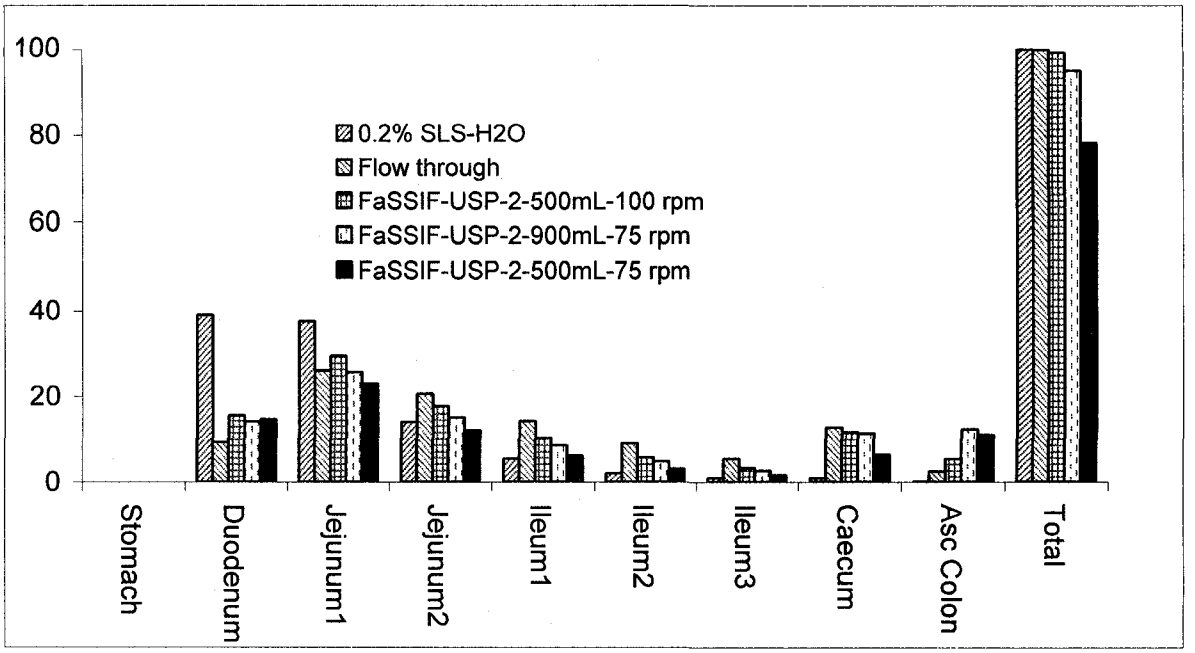

Figure 3-25 Montelukast sodium: Compartmental absorption patterns

3.3 Etoricoxib discussions

3.3.1 Solubility studies

The drug equilibrium solubility at pH 5.0 to 6.8, which is the duodenal and upper intestinal pH where most drug absorption takes place, indicate that etoricoxib does not meet the current FDA criteria for high solubility. The values are lower than the dose-relative solubility (0.48 mg/mL), which is the concentration corresponding to the highest dose strength of 120 mg in 250 mL. This might be due to the limitation in equilibrium solubility determination *in vitro*, which is static and does not take into account the dynamic nature of drug absorption *in vivo* (Yazdanian et al., 2004). Increasing the dose volume to 500 mL as suggested by Yu et al., (2002), would not change the classification of etoricoxib. However, at pH 1.2 and 4.1, the solubility of etoricoxib is high enough to enable it to be classified as highly soluble.

3.3.2 Intrinsic dissolution studies

Intrinsic dissolution is a rate phenomenon rather than a measure of equilibrium solubility; therefore it is expected to correlate closely with the *in vivo* drug dissolution dynamics than solubility (Yu et al., 2004). As such, Yu et al., (2004) proposed that an intrinsic dissolution rate of 0.1 mg/min/ cm^2 , be used as a class boundary for highly soluble drugs according to the BCS, taking into consideration the dose of the drug. Based on this observation, etoricoxib may be considered poorly soluble due to the low IDR at higher pH, although the rate is higher at low pH values.

The lower intrinsic dissolution rate in the two bile salt solutions (FaSSIF pH 6.5 and FeSSIF pH 5.0), is most likely caused by the higher pH of the media. In these media, etoricoxib has a lower solubility, and this may be supported by the fact that the natural surfactants are generally poor solubilizers compared with synthetic surfactants (Shah et al., 1989). The difference in the intrinsic dissolution rate (IDR) in SGF-SLS compared

with the USP-SGF could possibly be explained using the Stokes-Einstein equation for diffusivity given by equation 3-1:

$$D = \frac{k * T}{6\pi\eta r}$$ **Equation 3-1**

Where D = diffusivity, T = temperature, η = viscosity of the dissolution medium, r = radius of the drug molecule and k is the Boltzmann constant.

When all other factors are kept constant, r will be the radius of the micelle formed when SLS solubilizes the drug molecules. The effective diffusivity of a micelle is reduced compared to that of a drug molecule which is smaller than a micelle, resulting in a reduced diffusion coefficient, and a slower diffusion from the surface of the dissolving compact into the bulk medium (Hörter et al., 1997). This leads to a lower concentration in the bulk media at any given time point.

Crison et al., (1996) reported a decrease in the effective diffusivity with increasing concentration of sodium lauryl sulphate in the dissolution medium, when they determined the intrinsic dissolution rate of carbamezepine. Their findings support the observed difference in the intrinsic dissolution rates of etoricoxib in SGF with 0.25% SLS, compared with the high rate in SGF without SLS.

The large size of micelles obtained by particle size analysis is most likely aggregates formed due to the relatively high concentration of SLS above its critical micelle concentration (CMC). The CMC of SLS is reported to be about 0.03% (1.0417 mM) in 0.1 M HCL solution (Zhao et al., 2004), and in water the CMC of SLS is reported to be about 8 mM (or 0.23%) (Attwood, 1983; Ogino et al., 1987). From these values reported in literature, it is obvious that in 0.01 M HCl, the 0.25% concentration of SLS is above the CMC.

The dissolution rates observed in this experiment are typical of drugs that are of weak bases, which tend to have a high dissolution rate in acidic media, and their solubility and dissolution rate dramatically decreases as the pH increases. This may result to an *in vivo*

precipitation of the drug as it moves down the gastrointestinal tract, leading to low bioavailability, if the human effective permeability of the drug is also poor.

3.3.3 Dissolution studies

The high dissolution rate of etoricoxib in SGF-SLS following the dynamic pH change protocol can be attributed to its high solubility at low pH, which is likely due to the increased wetting and solubilisation of the drug particles by the 0.25% SLS in the medium as well as contribution from ionization since the pH is more than 2 pH units below the pKa of the drug. Dressman et al (1998) suggested that incorporating 0.25% SLS in the medium lowers the surface tension of the fluid to a level comparable to that experimentally determined by measuring the surface tension of gastric fluids taken from human subjects. A high intrinsic dissolution rate in the SGF-SLS media might also help to explain the fast rate of dissolution in the first 15 minutes in the flow through dissolution protocol before the medium was changed. During the test, the tablets were observed and complete disintegration within the first 5 minutes occurred.

A slow dissolution rate when the medium was changed to biorelevant medium at pH 6.5 and subsequently to pH 7.5 might be explained by the previously observed slow intrinsic dissolution rate of etoricoxib in biorelevant medium at pH 6.5. Bile salts had previously been reported to be slower in solubilising drugs compared with synthetic surfactants (Shah et al., 1989). The slow dissolution rate might also be due to the slower diffusion of the solubilised drug loaded in the mixed micelles formed by the lecithin and bile salts. Naylor et al., (1995) reported a nearly 100 fold reduction in the diffusivity of danazol in a lecithin/sodium taurocholate complexes compared with a sodium taurocholate solution alone. They attributed this to an increase in the diameter of the drug loaded micelles formed from about 40 to 2275Å.

In the USP Apparatus 2, the fast and complete dissolution rate of etoricoxib in 5 minutes in 0.01 M hydrochloric acid, pH 2.0, indicate that sink conditions prevailed in this

medium. The slower rate and a subsequent incomplete dissolution in FaSSIF-500 mL, FaSSIF-900 mL, phosphate buffer -900 mL and SIF-900 mL could be due to a lack of sink conditions. Based on the solubility of etoricoxib of ~ 0.14 mg/mL in these media and pH range, ~1.3 litres of dissolution media would be required to provide about the minimum volume (3 times the volume to reach saturation solubility) for sink conditions to prevail (The USP # 29). The 75 RPM agitation speed used appears to have provided adequate hydrodynamic conditions, since 100% drug was able to be dissolved in a medium where the drug is highly soluble, i.e. in 0.01 M HCl.

3.3.4 Similarity (f_2) and Difference (f_1) factors

A comparison was made between the dissolution profiles generated using the USP apparatus 2. The profile in FaSSIF-900 mL media volume was used as reference and the other profiles were compared with it. The results (Table 3-1) show that the dissolution profiles of etoricoxib in the USP-SIF and phosphate buffer are similar to that in FaSSIF-900 mL (f_2 =65.9 and 62.3, respectively) and the difference factor, f_1 were 3.3 and 4.0, respectively. The dissolution profile in FaSSIF-500 mL (the recommended volume) (Galia et al., 1998) is not similar to that performed using 900 mL volume (f_2 =44.8), but the difference is within acceptable limits (f_1=10.5). The major cause of the dissimilarity could be due to a lack of sink conditions at 500 mL volume compared with the 900 mL volume, although sink conditions do not exist in the 900 mL either. The profile from 0.01 M HCl could not be compared with other profiles, since dissolution was complete in 5 minutes, and the flow through method follows a completely different protocol.

3.3.5 Cell Culture permeability studies

The estimated value for the human effective permeability of 4.07 x 10^{-4} cm/sec suggests that the bioavailability of etoricoxib would not be affected by the intestinal wall permeability. Amidon et al (1995) demonstrated that the limit for >90% absorption

corresponded with a permeability of 2 x 10^{-4} cm/sec. The FDA and the EMEA guidance also define highly permeable as having a fraction of dose absorbed greater than 90%. These results therefore indicate that etoricoxib is a highly permeable drug whose bioavailability would be greater than 90%, and its bioavailability is reported to be 100% (Agrawal, et al., 2003). The permeability directional ratio (PDR), which is the ratio of BA/AB transport, was estimated to be 0.969. This indicates that etoricoxib is not a substrate for cellular efflux pumps. Yazdanian et al., (2004) suggested that drugs with a PDR ratio between 0.7 and 1.3 do not appear to have affinity for cellular efflux pumps.

3.3.6 Investigating possible *in vivo* precipitation under physiological conditions

Solubility and therefore dissolution may be the rate-limiting step in developing a dissolution test method for poorly soluble drugs. This is important when an IVIVC has to be established. It is therefore important to consider possible *in vivo* precipitation of a drug upon entry into the intestine. This is especially critical for weak base drugs that have a high solubility in the gastric medium but low solubility at high pH values.

Etoricoxib, a weak base has shown a high solubility and dissolution rate in the acidic environment of the stomach. Theoretically it is possible that as it moves down the GI tract and the pH rises, its solubility and dissolution rate decreases and it may precipitate out. This possible *in vivo* precipitation was investigated using a transfer model (Kostewicz, et al., 2003), in which etoricoxib solution in simulated gastric media is transferred by continuously pumping it into FaSSIF pH 6.5, and possible drug precipitation was monitored via concentration-time measurements.

The mean concentration-time profile of etoricoxib API dissolved in simulated gastric fluid and transferred to 500 mL of FaSSIF at the rates of 2.2 mL/min and 4.8 mL/min, shows a final concentration of the drug that appear to be higher than the equilibrium solubility predicted in both FaSSIF and SIF. No precipitation was observed to occur within two hours.

Precipitation of a supersaturated solution depends on nucleation and crystallization. The formation of the initial nuclei depends on the relative supersaturation, which is the difference between the actual concentration of solute before crystallization and its solubility limit. Mathematically, this can be expressed using equation 3-2:

$$Rs = \frac{C - Cs}{Cs}$$ **Equation 3-2**

Where Rs is relative supersaturation; C is actual concentration of solute before crystallization; Cs is its solubility limit. Von Weimarn (1926) recognized that stable nucleation rarely takes place when the value of relative supersaturation is less than 3. With a solubility of 0.14 mg/mL in SIF and a dynamic solubility concentration of ~0.2 mg/mL, etoricoxib in SIF has a relative supersaturation of ~0.42, which is far below 3, so we would not expect to see any precipitation occurring. This might help explain why the concentrations of etoricoxib in SIF appear to exceed its equilibrium solubility, which might as well be below its saturation solubility at the temperature at which the experiments were performed.

This also suggests that a high solubility of the drug can be achieved in the small intestine, if the drug undergoes complete dissolution in the stomach and is emptied into the duodenum. Since the concentration in solution is the driving force for absorption for drugs absorbed by passive diffusion (Kostewicz et al., 2003), it appears that the rate of absorption can be even higher than that predicted from aqueous solubility or media simulating the intestinal conditions.

3.3.7 Computer simulations using dissolution profiles

A large variability was observed in the individual plasma concentrations obtained from the 12 subjects whose plasma concentration-time data were used in this study. The inter-individual variability could be due to differences in physiological state at the time of the study. A student's t-test to compare whether there is any significant difference between

the observed AUC and simulated AUC showed that, at 95% confidence interval (95% CI), the AUCs obtained from all the dissolution data are not significantly different from the observed AUC ($p>0.05$). The C_{max} values obtained from the dissolution data in FaSSIF-500 mL, phosphate buffer and the USP-SIF, however, are significantly different from the observed mean C_{max} value ($p<0.05$).

The compartmental absorption patterns also shows that all the dissolution profiles predict nearly 100% of the drug absorbed in a similar fashion. These observations indicate that all the media tested could potentially be used to generate dissolution data, which can be used to simulate the *in vivo* profile for etoricoxib since they provide similar results.

3.3.8 Simulations as oral solution

A comparison of the simulated profiles generated using data from the flow through protocol;, the constructed 1 minute dissolution data and the solution model indicates that when taken as a tablet, etoricoxib is absorbed similar to a solution. This is most likely due to its fast disintegration and rapid dissolution in the gastric compartment. Any drug entering the small intestine is dissolved and seems to stay in solution. This argument can be supported by the fact that etoricoxib showed a high solubility in simulated gastric media, and when added to biorelevant medium at intestinal pH, it does not precipitate out of solution.

The compartmental absorption patterns also show that 79.5%, 79.7% and 78.4% of the drug is absorbed from the upper part of the gastrointestinal tract, when simulated using the dynamic dissolution protocol, controlled release 1 minute dissolution profile and as solution , respectively. Using this comparison, it can be argued that etoricoxib undergoes complete dissolution in the gastric compartment and is emptied into the duodenum in a dissolved state ready for absorption. The only rate limiting step to its bioavailability in such a case would be intestinal wall permeability.

The dynamic flow through protocol results in nearly 80% of the drug dissolved in the SGF in 15 minutes, before change to biorelevant medium. A comparison of the dissolution profile from the dynamic flow through protocol with the compartmental absorption patterns seems to support an argument that a significant amount of the drug dissolves in the gastric compartment and is emptied into the intestine in a dissolved state ready for absorption. Since the effective permeability of etoricoxib (4.07×10^{-4}) is high, it appears the dissolved drug is readily absorbed from the upper parts of the GI tract. Its bioavailability, therefore, seems to be regulated by the gastric emptying rate. A slower gastric emptying which is associated with the presence of food has no effect on the extent of absorption (Agrawal et al., 2003), it, however, decreases the rate of absorption resulting in an increase in t_{max} and a reduction in C_{max}. The flow through compartmental absorption pattern appears to represent an absorption pattern when the drug is taken in a fasted state.

3.3.9 Statistical analysis

The regression analysis indicate that the flow through dynamic protocol, FaSSIF-900 mL and the 0.01 M HCl provides the best *in vitro/in vivo* correlation ($r^2 = 0.90, 0.899, 0.898$). The sums of square error of prediction (SSE = 0.195, 0.195 and 0.97, respectively), the root mean square error (RMSE = 0.101, 0.101, 0.102, respectively) are the lowest compared with the other dissolution profiles.

Simulations using a controlled release oral solution (where 100 % is dissolved in 1 minute), also provided a good correlation ($r^2 = 0.90$, SSE = 0.193, RMSE = 0.101). This similarity supports the assumption that etoricoxib behaves like an oral solution when administered as tablets. Due to its rapid disintegration, it appears that the drug undergoes rapid dissolution in the presence of the hydrochloric acid in the stomach, and is emptied into the intestine in a dissolved state.

Dissolution profiles from the USP-SIF, phosphate buffer, and FaSSIF-500 mL provide weaker correlations (r^2=0.676, 0.668 and 0.593, respectively). The SSE (SSE =0.613, 0.634 and 0.820, respectively) and the RMSE (RMSE =0.180, 0.183 and 0.208, respectively) are also high in all the three profiles. This is probably due to a slower *in vitro* dissolution rate observed from the three dissolution profiles, which would result in the drug taking a longer time to complete dissolution.

The percent prediction error (PE) statistics show that all the six dissolution profiles over-predicted the AUC to a similar extent, while only FaSSIF-500 mL, the phosphate buffer and the USP-SIF in the USP apparatus 2 under predicted C_{max} to an extent greater than the flow through dynamic protocol, the biorelevant medium (FaSSIF-900 mL) or 0.01 M HCl using the USP apparatus 2. This suggests that phosphate buffer and the USP-SIF may not be the best choice of media, while using FaSSIF at 500 mL may not be the right choice of volume, for *in vitro* testing of etoricoxib, when intending to establish IVIVC.

When simulated as an oral solution, the prediction error statistics are comparable with either the flow through or FaSSIF-900 mL dissolution. This similarity again supports the argument that when taken as a tablet, etoricoxib behaves like an oral solution.

3.4 Etoricoxib conclusions

The simulations showed that etoricoxib is completely absorbed when administered as a tablet and as solution. The Caco-2 cell culture permeability results indicated that the drug is highly permeable. Although solubility studies showed that the drug is poorly soluble across the GIT pH range (1.2-7.5), the experiments showed that if the entire dose of etoricoxib is dissolve in gastric media, and added to FaSSIF it continues to stay in solution due to solubilization by the bile salts and lecithin present in the media. This most likely indicate that in case of *in vivo* dissolution in the gastric compartment, the drug would continue to stay in solution when emptied into the small intestine due to the

presence of the native surfactants in the GIT and sink conditions can be maintained by the rapid absorption of the solubilized drug.

Based on the observation of the behaviour of the drug, it would appear that the bioavailability of etoricoxib is not limited by the dissolution process. Its permeability is higher than the suggested cut-off mark of 2×10^{-4}, which enables the drug to be considered highly permeable. A parameter sensitivity analysis indicated that the extent of absorption, which is measured by the AUC, is not affected by a variation in effective permeability value ranging between 1 and 8×10^{-4} cm/s.

Although its oral absorption is complete resulting into 100 % bioavailability, etoricoxib only complies with the BCS definition of high solubility at low pH. The BCS considers aqueous solubility, at least 85% dissolution in 30 minutes across a pH range of 1-7.5 in buffers and a bioavailability of >90%, in order to place a drug into class I (Amidon et al., 1995). It does not, however, take into consideration the behaviour of drugs that are weak bases, are highly soluble in the gastric media, and when emptied into the duodenum continue to stay in solution.

While the behaviour of etoricoxib is typical of a BCS class I drug, from the observations made, it appears that following the strict definition of the BCS, etoricoxib may only fit in the proposed intermediate solubility class suggested by Yu et al (2002), Polli et al., (2004) and Yazdanian et al., (2004), for acidic and basic drugs that are highly soluble at either physiological relevant pH 1.2 or 6.8.

Simulations show that etoricoxib behaves like an oral solution. Statistics indicate that for better simulations and hence IVIVCs, the flow through protocol, the biorelevant media (FaSSIF at 900 mL) and 0.01 M HCl in the USP apparatus-2 are the better choice of method and or media, compared with the USP buffers or biorelevant media at 500 mL volume.

In this project, the properties of etoricoxib studied, that are related to the biopharmaceutics classification system (BCS) places the drug to be a BCS class II drug, because the intermediate class proposed has not been adopted by the regulatory agencies e.g. the FDA. It is likely that for different dose strength, differences in composition and/or manufacturing processes may not have an effect on the rate and extent of absorption. If there are any such differences, they are likely to be detectable by carefully designed comparative *in vitro* dissolution tests. Also in view of its therapeutic use, its wide therapeutic index and simple pharmacokinetic properties, a biowaiver for immediate release (IR) etoricoxib solid oral drug products can be scientifically justified, provided that the dosage strengths contain only those excipients reported on the drug packaging by the manufacturer.

3.5 Montelukast sodium discussions

3.5.1 Solubility in different media

The increased solubility of montelukast sodium in SGF-SLS compared with SGF without SLS is likely due to better wetting and solubilising effect of the surfactant (SLS). The high solubility of montelukast sodium in biorelevant media compared with the corresponding blank buffers at the same pH can be attributable to the poor wetting properties of the drug particles by the buffer systems as a result of the high lipophilicity of the drug. The highest solubility was observed in biorelevant media at pH 7.5, and is even higher in media prepared using high quality bile salts (4.69 mg/mL) compared with media prepared using crude bile salts (3.37 mg/mL). This difference could be due to the difference in the purity of the bile salts and lecithin used in the study.

Although the general trend indicates that the solubility of montelukast sodium is pH dependent, these results also show that the presence of the bile salts has a significant effect on the solubility of montelukast sodium, within the pH range at which these experiments were performed. Based on the entire physiological pH range of 1.2 to 7.5, montelukast sodium can be considered a poorly soluble drug and can best be described as a BCS class II or Class IV drug. It is expected that its dissolution behaviour *in vivo* varies as the drug moves down the GIT, with faster dissolution taking place further down in the gut where the pH is higher and favourable.

3.5.2 Dissolution tests

A high dissolution rate in SGF-SLS (0.1%) is most likely due to the increased wetting and solubilisation of the drug particles by the SLS (0.1% w/v) present in the medium. Enhanced dissolution rate at high pH could be due to the combined effect of bile salts/lecithin as well as the pH, since montelukast solubility was found to be high at pH 7.5. The enhanced dissolution of montelukast sodium in biorelevant media is most likely

due to improved wetting and solubilization by the bile salts and lecithin. Galia et al., (1998) reported an increase in dissolution of poorly soluble drugs in the presence of bile salts and lecithin. These changes suggest that *in vivo*, the dissolution rate of montelukast sodium would also change as the drug particles travel down the gastrointestinal tract. Complete dissolution would be expected to take place in the distal part of the small intestine, which will result into absorption taking place throughout the GIT.

A higher percent drug dissolved was observed in FaSSIF-500 mL at 100 RPM (88.9%) compared, with 69.4% when 75 RPM was used. This indicates that the agitation speed has an impact on the extent of dissolution in FaSSIF at pH 6.5. The 100 RPM appears to provide better hydrodynamic conditions compared with 75 RPM. However, incomplete dissolution at both speeds might be due to a lack of sink conditions. Maintaining sink conditions requires that the volume of dissolution media be at least 3 times greater than the volume at the saturation point of the drug contained in the dosage form being tested (The USP # 29). At 0.02mg/mL solubility in FaSSIF, a 10 mg dose, if completely dissolved in 500 mL of medium would give a concentration of 0.02 mg/mL which equals the saturation point. Based on the solubility of montelukast sodium in FaSSIF, it appears that at 500 or 900 mL sink conditions does not exist, which is supported by the results that an increase in the volume of dissolution medium to 900 mL did not make any significant difference.

The *f-test* for similarity and difference however indicate that the dissolution profile in FaSSIF-900 mL is similar to the dissolution profile in FaSSIF-500 mL-100 RPM (f_2 =54.6 and f_1 = 5.0), while the dissolution profile in FaSSIF-500 mL-75 RPM is different from that in FaSSIF-500 mL-100 RPM though the difference (f_1) is within acceptable limits (f_2 =48.0 and f_1 = 6.9). The dissolution profiles in phosphate buffer and the USP-SIF fail to match the profiles in biorelevant media, whereas dissolution in water with 0.25% SLS, is so fast that it would not represent the *in vivo* dissolution behaviour of montelukast sodium under physiological conditions.

3.5.3 Computer simulations

The dissolution data obtained were used as input functions into Gastroplus™ to simulate the *in vivo* profile of Montelukast sodium obtained from a clinical study reported by Zhao, et al., (1997). Different values for the oral bioavailability of montelukast sodium have been reported in literature. In the elderly and young healthy subjects bioavailability (BA) values were reported as 61% and 62% (Zhao et al., 1997), while Cheng et al (1996) reported bioavailability values ranging between 58-66% in healthy male and female subjects.

Montelukast sodium is reported to undergo extensive hepatic metabolism, with the major pathway for excretion being through the bile (Balani et al., 1997; Chiba et al., 1997). The major enzymes responsible for montelukast sodium metabolism in the liver are cytochrome P450 3A4 and 2C9. These enzymes are reported to be expressed at various sites throughout the gastrointestinal tract as well.

Using intestinal microsomal preparations, Thumel et al., (1997) and De Waziers et al., (1990), found that the expression of CYP3A4 shows a significant gradient throughout the GI tract, with highest levels in the duodenum and jejunum, which are the major sites of drug absorption, while the expression level is lower in the ileum. The metabolism rate of several CYP3A4 substrates by the upper intestinal microsomes is comparable to that in the liver (Lampen et al., 1998) or might sometimes even be higher (Paine et al., 1997).

The presence of these enzymes in the gut might have an impact on the overall bioavailability of montelukast sodium, through first pass extraction and gut wall metabolism. There are no reports which have investigated the effect of co-administration with known inhibitors of cytochrome P450 3A4 (e.g. ketoconazole or erythromycin) or 2C9 (e.g. Fluconazole), on the bioavailability of montelukast sodium.

Based on the reported bioavailability of montelukast sodium, first pass extraction was taken into consideration during the simulations. Comparisons were made between the

observed and simulated profiles, using a 38% first pass extraction. Using this value lowered the bioavailability to literature values and the observed and predicted values show a better match. A comparison of the simulated profiles from the flow through cell dissolution with and without first-pass extraction correction show that in order to better predict the *in vivo* behaviour of montelukast sodium from dissolution data, first pass extraction needs to be taken into consideration.

All simulations were therefore performed with the dissolution data obtained, using a 38% first pass extraction correction. The simulated profile from the surfactant solution (0.25% SLS in water) however over-predicted the C_{max}, while the in the USP-SIF and phosphate buffer (less than 10% of the drug dissolved), resulted in profiles close to baseline.

A student's t-test to compare whether there is any significant difference between the observed AUC and simulated AUC showed that, at 95% confidence interval (95% CI), AUCs simulated using data from Flow through cell, FaSSIF-500 mL-100 RPM, FaSSIF-900 mL, 75 RPM and H_2O-0.25% SLS are not significantly different from observed ($p>0.05$). Only the C_{max} from H2O-0.25% SLS is significantly different from observed ($p<0.05$). The profiles from SIF and Phosphate buffer are not comparable with the observed *in vivo* profile.

Regression analysis indicated that a better correlation with *in vivo* profiles can be achieved using the flow through dissolution data ($r^2 = 0.979$ and SSE= 0.006). Good correlations can also be obtained using the FaSSIF-500 mL-100 RPM ($r^2 = 0.834$ and SSE= 0.053) and FaSSIF-900 mL ($r^2 = 0.756$ and SSE= 0.057). Correlations with FaSSIF-500 mL at 75 RPM, and in water with 0.25% SLS is rather weak. Dissolution in the USP-SIF and phosphate buffers provided extremely weak correlations.

The simulation results indicated that dissolution tests using biorelevant media are able to predict the *in vivo* behaviour, and the flow-through dissolution tests following dynamic pH change protocols are able to mimic *in vitro* the environmental changes that impact the solubilization and *in vivo* dissolution of montelukast sodium better.

The USP 29 (chapter 1088) and the FDA guidance to industry (FDA-CDER, 1997) describe four different levels of IVIVC. Level-A IVIVC represents a point-point relationship between the *in vitro* dissolution curve and the entire *in vivo* input rate. A level A is considered as the highest level of correlation. From these observations, the flow through dissolution tests following the dynamic pH change protocol appears to predict the *in vivo* dissolution as required for a level-A correlation.

The percent prediction error showed that the profile in H_2O with 0.25% SLS over-predicted C_{max} by 67.4 percent, although it predicted the AUC to within 0.4 %. The flow through cell and FaSSIF-500 mL-100 RPM data under-predicted AUC by 1% and 1.7 %, respectively, while they over-predicted the C_{max} by 18.2%, 11.6 %, respectively. FaSSIF-900 mL under-predicts AUC and C_{max} by 6.9% and 3.6 %, respectively. FaSSIF-500 mL-75 RPM under-predicts both AUC and C_{max} by 24.5 and 11.2%, respectively. The sums of squares (SSE) and RMSE is the lowest for the flow through profile, followed by the FaSSIF-500 mL-100 RPM, indicating that these two dissolution profiles could be used to establish IVIVC. Phosphate buffer and the USP-SIF both have the highest percent prediction error, indicating that they were not suitable media for dissolution testing of montelukast sodium when attempting to establish an IVIVC.

The compartmental absorption patterns show that the absorption of montelukast sodium occurs throughout the GIT, indicating that dissolution might be the rate liming step to its bioavailability. The absorption pattern in water with 0.25 % SLS were quite different from other profiles. More than 90 % was absorbed from the upper part of the GI tract. The simulated profile and absorption pattern obtained by using the dissolution profile in water-0.25% SLS solution support the argument that, indeed the absorption of montelukast sodium is dissolution controlled. A fast *in vitro* dissolution rate results into a fast absorption, but from the observations made, dissolution *in vivo* is not as fast as portrayed by the profile in water-SLS medium.

3.6 Montelukast sodium conclusions

With respect to solubility and permeability, the BCS class boundary is considered too conservative (Yazdanian et al., 2004). Permeability class boundaries are based on the estimate of the fraction dose absorbed in humans or measurements of rate of mass transfer across intestinal membranes (Yu et al., 2002). The regulatory guidance defines a drug to be highly permeable when the extent of absorption in humans is 90% or more in the absence of any documented instability in the GIT or whose permeability has been determined experimentally (FDA/CDER, 1997).

Both Caco-2 and MDCK cell line permeability studies did not yield any results that would give permeability estimate for montelukast sodium. The MDCK cell lines were used because they are generally thought to have a leakier tight junctions compared with Caco-2 cell lines, based on their lower TEER value. This might however work well for drug transported via the paracellular route or hydrophilic drugs. The failure to see any drug transported in both cell lines might probably be due to the high molecular weight of montelukast sodium (608.18), which is considered too high to be transported by the paracellular route, or alternatively the tight junctions formed by the cell monolayer were too tight for montelukast to be transported by this route.

Transport study using cell monolayers is static in nature; as such there is a possibility that there was lack of sink conditions that would pull the absorbed drug molecules away from the site of absorption as would occur *in vivo* due to blood circulation. Due to the high lipophilicity, the drug molecules that gain entry into the enterocytes may simply stay within the enterocytes and are therefore not transported across the basolateral membrane.

It is also possible that montelukast transport across the enterocytes is facilitated by transporters not expressed in the two cell lines used. However, the permeability value of 9.49×10^{-4} cm/s obtained from ADMET predictor™ and used in the simulations are high enough to consider montelukast sodium a highly permeable drug. However, the solubility data allows montelukast sodium to be considered poorly soluble.

The simulations showed that montelukast sodium is completely absorbed throughout the GIT. Good correlations with *in vivo* dissolution can be established using the flow through cell dissolution following the dynamic pH change protocol, as well as biorelevant media in the USP Apparatus 2.

The fact that it undergoes extensive pre-systemic metabolism, makes its bioavailability appear lower that the actual fraction of dose absorbed. Its bioavailability appears to be dissolution controlled and not permeability limited. Its dissolution behaviour which seems to be pH dependent supports this argument. As such, montelukast sodium would best be classified as a BCS class II drug, since simulations showed that, if the drug is dissolved, it is all absorbed.

More research is required especially to estimate the effective permeability using methods, other that the *in vitro* cell culture techniques to help support the placement of montelukast sodium in the right BCS class.

3.7 Summary and general discussions

In this study, attempts were made to develop dissolution test methods that can be used to forecast the *in vivo* behaviour of the two drugs used in the study, and IVIVCs using computer simulations were established. Based on the observations on the behaviour of the two drugs, an attempt was also made to scientifically justify placing the two drugs in their corresponding BCS classes. The BCS was developed based on the two fundamental properties controlling the rate and extent of oral drug absorption, i.e. solubility and gastrointestinal permeability (Amidon et al., 1995).

Solubility studies for the two drugs were performed using the USP buffer and biorelevant media. It was observed that the solubility of etoricoxib is pH dependent, with a high solubility at low pH and the solubility decreases as the pH increases. Bile salts and lecithin appear to have no impact on the equilibrium solubility of the drug. It was,

however, noticed that when a solution of etoricoxib dissolved in simulated gastric fluid at low pH and slowly added to BDM at high pH, it continues to stay in solution without precipitating for at least 2 hours. This is thought to be significant *in vivo*, should the drug dissolve completely in the stomach and slowly be emptied into the intestine where the pH is higher.

Montelukast sodium also shows a pH dependent solubility, with a low solubility at low pH. Its solubility increases as the pH is increased and its solubility was observed to be higher in BDM at higher pH values compared with the corresponding blank buffers of the same pH.

Dissolution tests were performed using the USP Apparatus 2 and using the flow through cells following dynamic pH change protocols. For both drugs, it was observed that the dissolution behaviour in the flow through cells following the dynamic pH change protocol followed the solubility behaviour of the drugs in the respective medium and pH. In the USP apparatus 2, the dissolution behaviour of etoricoxib in BDM was similar to that in the USP buffers. For montelukast sodium, there was a significant difference in the dissolution behaviour when the profiles in BDM were compared with that in the USP buffers. Better dissolution was observed in BDM, and hydrodynamics conditions were observed to cause a significant increase in the extent of dissolution.

Caco-2 and MDCK cell lines were used to estimate the apparent permeability of the two drugs *in vitro.* Permeability data was obtained for etoricoxib using the Caco-2 cells. The permeability values obtained for the A/B and B/A showed that etoricoxib is not a substrate for P-glycoprotein (Pgp) cellular efflux pumps, and it appears that its absorption is mainly by the passive transcellular route. The values for apparent permeability converted to human jejunum effective permeability using the GastroPlus™ converter utility showed that etoricoxib is a highly permeable drug.

There was no apparent transport across the cell monolayers observed for montelukast sodium using both the Caco-2 and MDCK cell lines. This is thought to be due to the high

lipophilicity of the drug, such that the drug molecules that enter the enterocytes may simply get trapped within the enterocytes and are therefore not transported across the basolateral membrane, but this was not however proven. The human jejunum effective permeability for montelukast sodium was therefore estimated *in silico* using the ADMET Predictor™ computer software.

There was no significant difference observed in the simulated profiles of etoricoxib using the flow through cell dissolution testing following the dynamic pH change protocol, 0.01 M HCl and FaSSIF-900 mL in the USP Apparatus 2. The USP buffers and 500 mL FaSSIF however showed a significant difference between the simulated and the observed profiles. The absorption patterns indicated that the drug is completely absorbed, with most absorption taking place in the upper parts of the GI tract. Etoricoxib bioavailability does not appear to be limited by permeability or the dissolution process. The drug behaves like a BCS class 1 drug, but due to the conservative nature of the BCS class boundary with respect to solubility, etoricoxib seems to best fit in the proposed intermediate class.

For montelukast sodium, the flow through dissolution testing following the dynamic pH change protocols simulated the *in vivo* profile better compared with dissolution profiles from the USP Apparatus 2. Literature data shows that montelukast sodium is a substrate for the CYP3A4 metabolic enzyme, which is also found in the gut walls as well as the liver. It appears therefore that the drug undergoes a considerable pre-systemic metabolism both in the gut walls and the liver, whose net effect is lowering its bioavailability. Better simulations required taking into account first-pass extraction.

Simulations showed that montelukast sodium is completely absorbed, and its absorption takes place throughout the GI tract. Its bioavailability appears to be limited by the dissolution process and not permeability. Based on these observations, montelukast sodium appears to best fit in the BCS class 2.

For both drug products, the flow through dissolution testing following a dynamic pH change protocol seem to be better in establishing an *in vitro/in vivo* correlation compared with dissolution testing using the USP Apparatus 2. This conclusion is supported by the statistical analysis results which show a better correlation, when the simulated *in vivo* profiles whose shape appears to be best determined by the *in vitro* dissolution data is compared with the observed *in vivo* plasma concentration-time profile. Further research is however needed to investigate the relationship between absorption patterns and dissolution behavior of drugs.

In conclusion, the use of *in vitro* tests in combination with *in silico* simulations using computer software like GastroPlus™ seems to be revolutionizing the way *in vitro/in vivo* correlations should be perceived and performed. In a clinical study, a combination of physiological factors and physiochemical characteristics of the drug work for or against each other *in vivo* in order to enable measurement of the plasma-time concentration of an orally administered drug.

Due to the complexity of the drug absorption process and the complexity of the GI tract, there is no single *in vitro* test that can precisely mimic the *in vivo* behaviour of oral drugs. Rather, each *in vitro* test only gives an idea of the *in vivo* behaviour. Physiologically based computer simulation programs that uses several physiochemical measurements and physiological characteristics to simulate the *in vivo* behaviour of orally administered drugs, in my view is the way forward in predicting the *in vivo* behaviour, and hence establishing *in vitro/in vivo* correlations.

CHAPTER -4

4 ACHIEVEMENTS AND SUGGESTION FOR FUTURE RESEARCH

4.1 Achievements

The conclusions and achievements of this research project include:-

1. The establishment of an *in vitro* dissolution test protocol that can be used to predict the *in vivo* drug behaviour in combination with Gastroplus™.
2. An *in vitro* proof that weak bases can continue to stay in solution when added dissolved into buffers and BDM's of higher/unfavourable pH values.
3. The development of a transfer model that can enable the reclassification of weak bases which are borderline BCS class 1 drugs.
4. The *in vivo* behaviour of drugs whose bioavailability are dissolution rate controlled are best predicted using dissolution profiles obtained by the flow-through cell following a dynamic pH change protocol and Gastroplus™.
5. Level A IVIVC can be established using the developed *in vitro* dissolution test methods and *in silico* simulations as performed with Gastroplus™.

4.2 Suggestions for future research

The BCS was developed to provide a framework upon which the oral drug absorption can be predicted, based on solubility and permeability characteristics of the drug candidate (Amidon et al., 1995). The BCS does not however take into consideration the solubility behaviour of certain drugs that show high solubility at either extreme pH conditions. This was found appear to have a great implication for especially weak bases that are highly soluble in acidic environment and when added to BDM, continue to stay solubilized. Based on the strict definition of the BCS, drugs could end up being placed into a wrong BCS class where they may not actually belong. It is therefore necessary to perform such dynamic dissolution studies with more drugs that are weak bases, because such kind of

test protocols could help scientists gain more understanding on the *in vivo* behaviour of these drugs, which could be used to find the right BCS classification.

This study showed that a combination of *in vitro* dissolution testing and *in silico* simulations can be used to scientifically justify the request for a biowaiver for a drug candidate. More studies are required using other drugs that are weak bases, because the outcomes from such studies may be useful to the scientific community in justifying biowaiver for a drug. The pharmaceutical industry could benefit by using the results in requesting biowaivers from *in vivo* bioequivalence testing in humans for certain drugs, resulting into shorter drug development time, saving money and possibly causing a reduction in the cost of drugs. When more drugs become affordable, this could result to an improvement in the quality of life of patients.

The developed dissolution test method using the USP apparatus IV, flow through cell following a dynamic pH change protocol was proven to be feasible in predicting the *in vivo* behaviour of montelukast sodium, a poorly soluble drug whose bioavailability appear to be dissolution rate controlled. More extensive studies following similar protocols are necessary using other drugs that show similar behaviour like montelukast sodium, in order to validate the developed dissolution test method. This might lead to the development of a universal *in vitro /in silico* test protocol that may be useful to the industry, thus shortening the overall drug development time.

The outputs obtained from the computer simulations include the simulated oral plasma profile and compartmental absorption patterns. Further research is therefore required to evaluate how the compartmental absorption patterns obtained from simulations using the *in vitro* dissolution profiles be used in designing dosage forms that can improve bioavailability.

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APPENDIX 1

1 USING GASTROPLUS TO PERFORM SIMULATIONS

1.1 Introduction

GastroPlus™ uses the Advanced Compartmental and Transit (ACAT) model to simulate the gastrointestinal absorption and pharmacokinetics for orally and intravenously administered drug products in humans and in some species of animals like the dog, cat and monkey. The minimum *in vitro* and *in vivo* data required in order to be able to perform simulations include:

1. The pH solubility profile of the drug covering the gastrointestinal pH profile.
2. Dissolution profile.
3. Permeability data, which can be obtained from cell cultures, rat, dog or directly from humans.
4. The pKa(s) of the drug.
5. At least an oral and an intravenous clinical data set for one dose strength for each route of administration.

Additional data that are required but may not be necessary in the simulations are:

6. The particle size distribution of the active pharmaceutical ingredient (API).

Once all the required data is obtained, one is then ready to perform the simulations. The text that follows provides a simplified step-by-step procedure one can follow to be able to us the program to run a simulation.

1.2 Starting GastroPlus™ to create a database and enter drug information.

Initially when GastroPlus™ is started, the user logs in and a window is displayed, from which the user creates the drug database (Figure A-1).To create a new drug database, the user selects "**File-new Drug database",** and save the database with name of your choice. In the new database created, one drug record, with the default name **"New Drug"** is

created. A new file with the name of the drug of choice can be created by clicking on **"Database"** in the new drug database created and selecting **"create new drug record"**. A file name is then given for the new drug record and is saved in the new drug database.

In the compound tab (Figure A-2), information pertaining to the new drug such as dose, solubility and the reference pH at which the solubility was measured, effective permeability, drug particle density, molecular weight and molecular formula are entered. When the pKa of the drug is known, the user can click on the button labelled **"pKa table"**, and a window pops up where the pKa of the drug can be entered. Optionally, if the drug particle size distribution is known, the mean particle radius can be obtained and can be entered by clicking on the button **"Particle Radius"**, a window pops up where the particle radius is entered.

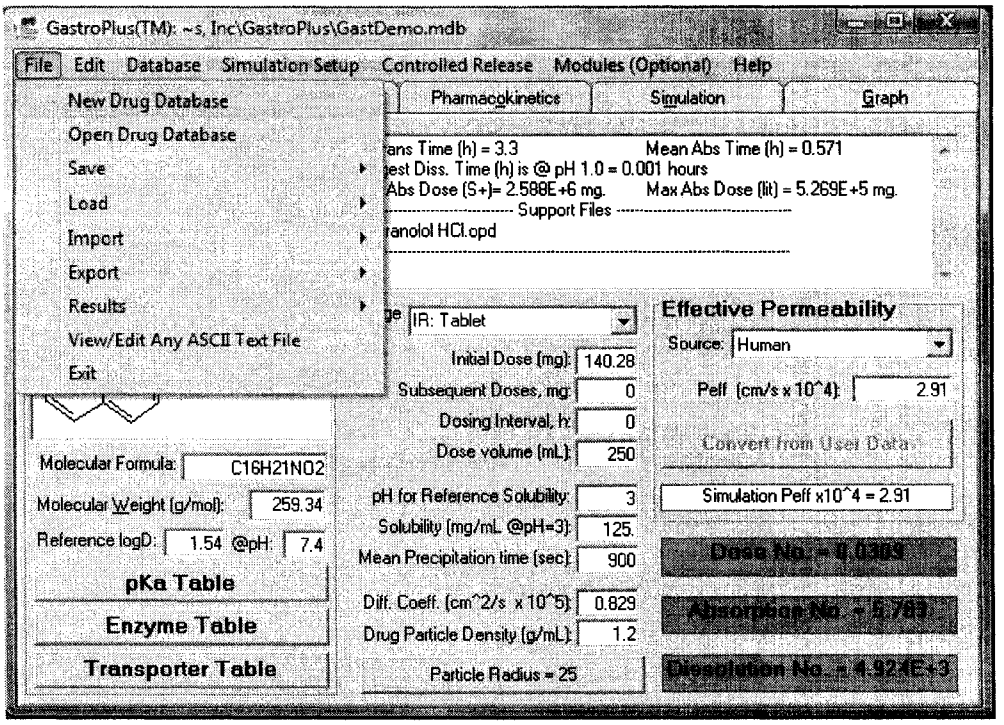

Figure-A 1 Creating a drug database.

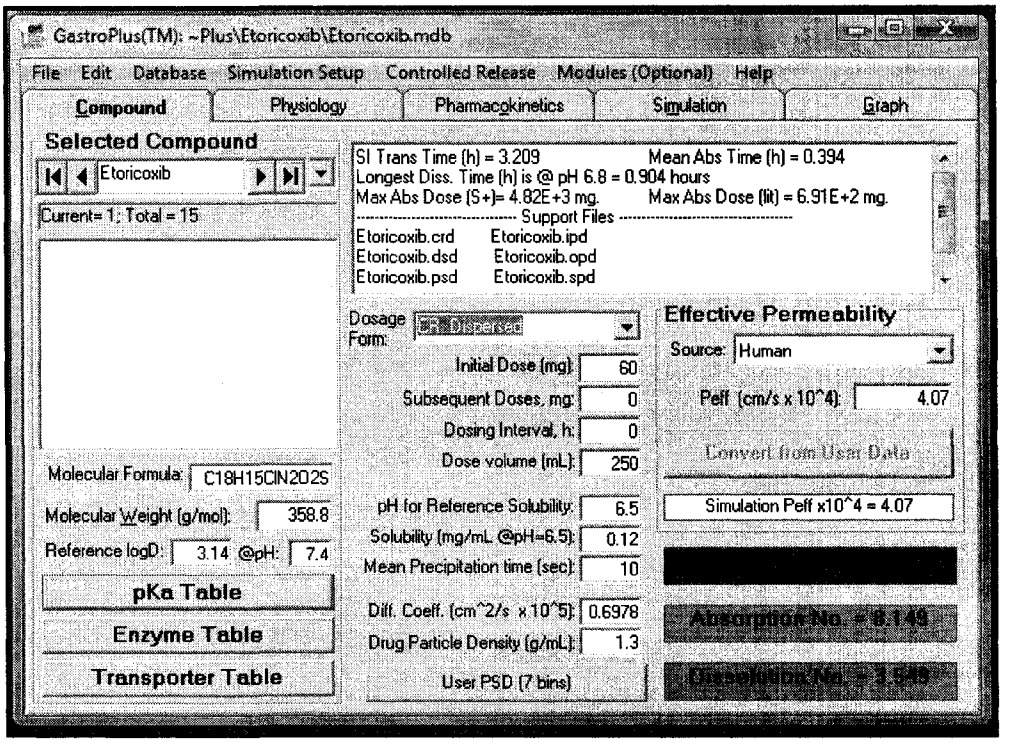

Figure-A 2 The compound tab

1.3 Data entry into the database created

The next step is entering the necessary data needed for simulations. The minimum four entries to be made for simulations using the dissolution data include:

- Dissolution profile (entered as controlled-release vs. time profile).
- Drug particle size distribution.
- Intravenous plasma concentration-time data.
- Oral plasma concentration-time data.

Note that the correct units of time and concentrations are selected when making each entry. Figure A-3 shows the window that can be used for selecting the options to make each of the entries mentioned above. Before exiting the window for each entry, the file needs to be save, by clicking **"File-Save"**, GastroPlus™ automatically provides the name for each of the files, similar to the drug record name, but with file extensions corresponding to each entry.

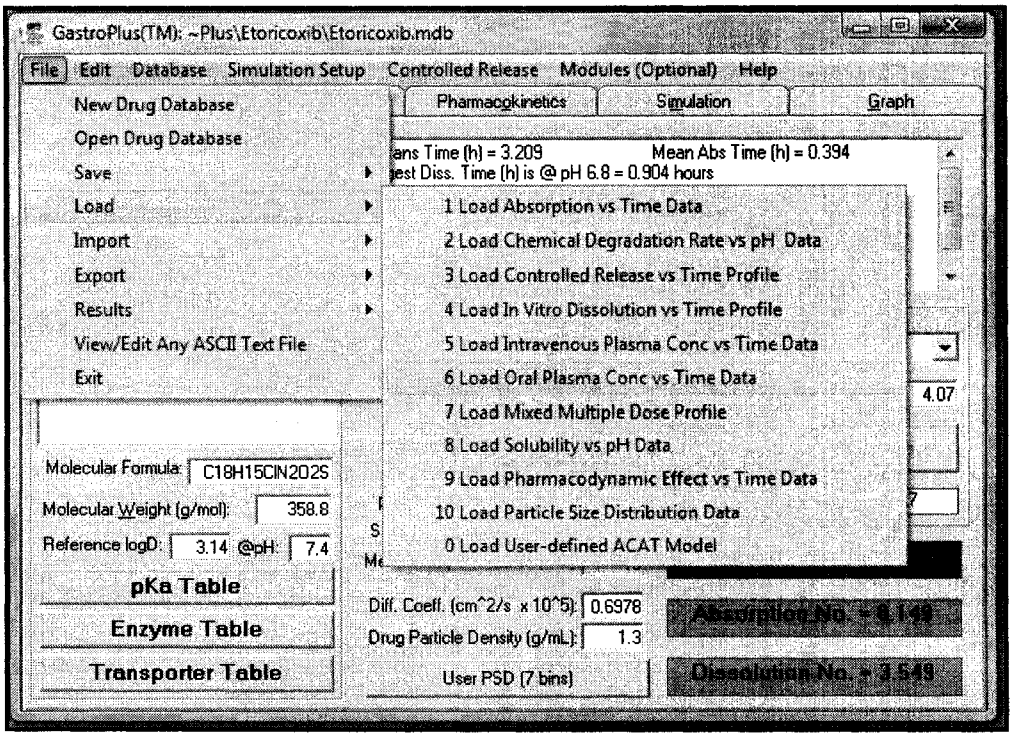

Figure-A 3 Loading files

1.4 Compartmental Modelling

Before any simulations can be done, the drug's disposition kinetics needs to be defined. If the disposition kinetics can be described by more than one compartment, the inter-compartmental rate constants (k_1k_2, k_2k_1 etc) need to be estimated. The modelling can be performed using the PKPlus™ option in GastroPlus™. Figure A-4 shows the window to use in order to obtain the rate constants. Click on **"Modules (Optional)"** and select **"PKPlus™"** from the menu. A window similar to Figure A-5 pops up. You can enter the intravenous data manually and select redraw. If you had earlier entered the data using the data entry option, selecting **"File-open"** from the top left corner of the window will pop another window similar to Figure A-6.

In figure A-6, what you will need to enter is the **"Dose"** and the **"Body weight"**, and if it was **not** an **iv bolus**, but an infusion, enter the infusion time, and click **"solve"**. The program will perform the modelling and at the end a display will show, giving the best compartmental model with the values of clearance, volume of distribution and inter-compartmental rate constants (Figure A-6). The user then has the option of copying the pharmacokinetic parameters obtained or exporting directly to the drug record. The export method is preferred, so the user needs to select **"File-export"**, and the necessary rate constants are exported directly into the pharmacokinetic tab of the drug record, and can be checked by selecting the Pharmacokinetic tab (Figure A-9). After this, you are now ready to perform simulations.

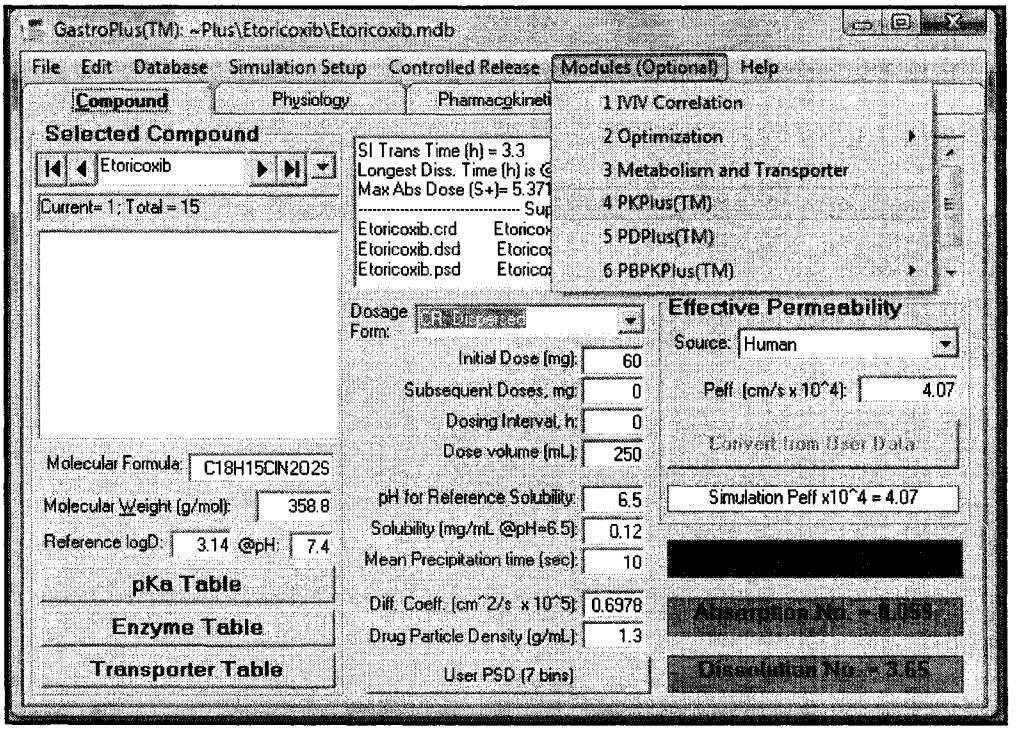

Figure-A 4 selecting optional modules

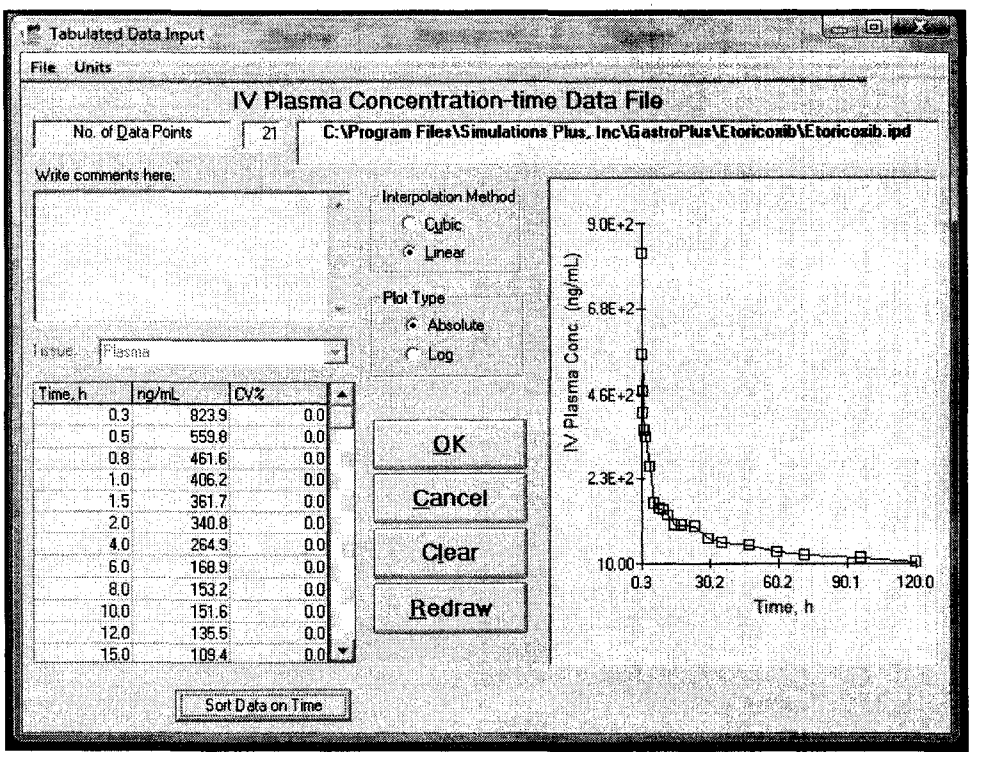

Figure-A 5 Data input screen for compartmental modelling

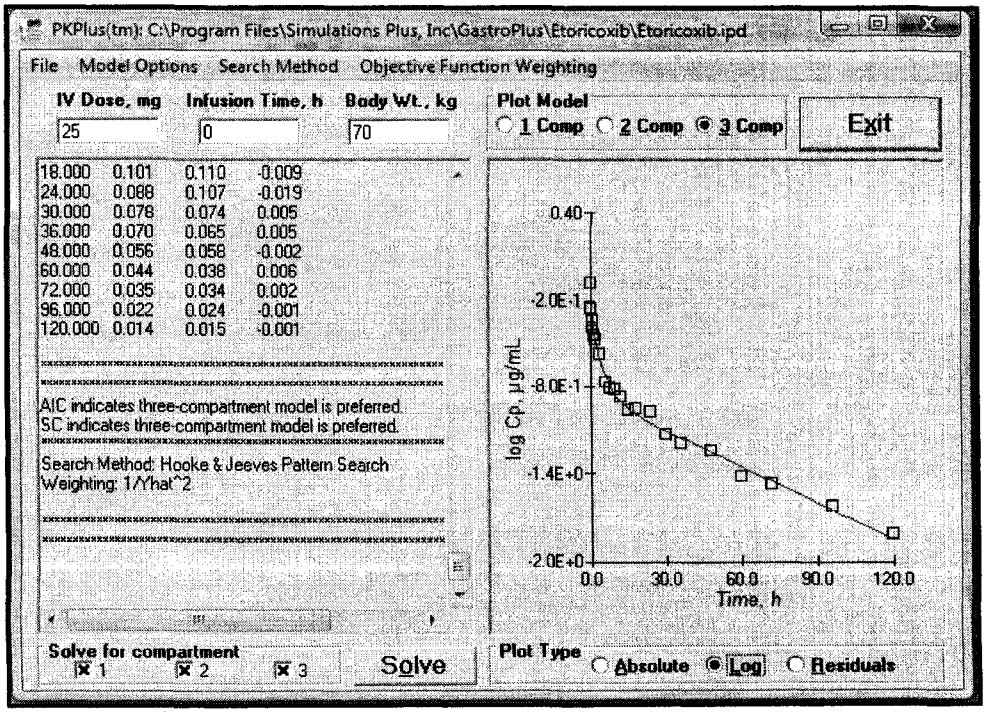

Figure-A 6 Completed compartmental model

1.5 Setting up to Perform simulations

When ready to start the model building, you need to go back to the compound tabs (Figure A-1). Ensure that all the information previously entered are all correct, then select the **"CR-Dispersed"** from the **"Dosage form"** drop-down menu, proceed to the physiology tab (Figure A-7). The Peff, ASF, pH, Transit times, C1-C4, Qh and "percent fluid in comp." values can be left at their default values. Only the physiology selector and the absorption model selector can be change from the "Physiology and ASF Model" dropdown menus. The different options are displayed in Figure A-8.

After selecting your options, move to the Pharmacokinetic Tab (Figure A-9).All the pharmacokinetic parameter on the left side of the window will have been imported from the PKPlus™, so there will be no entries to make. Ensure that the values for Clearance, Vc and the inter-compartmental rate constants are present. Enter the average weight of the individuals obtained from the pharmacokinetic study, the percent of drug bound to plasma proteins and percent unbound if known. First pass extraction, if fixed or estimated from the pharmacokinetic study. If unknown, enter zero (0). On the right side of the screen, enter the values of the drug bioavailability, Cmax, Tmax and AUC if known. If there is any unknown value, leave it as zero. For metabolism/transporter scale factor, the default values are okay. After crosschecking that everything is okay, the user can then move to the simulations tab (Figure A-10).

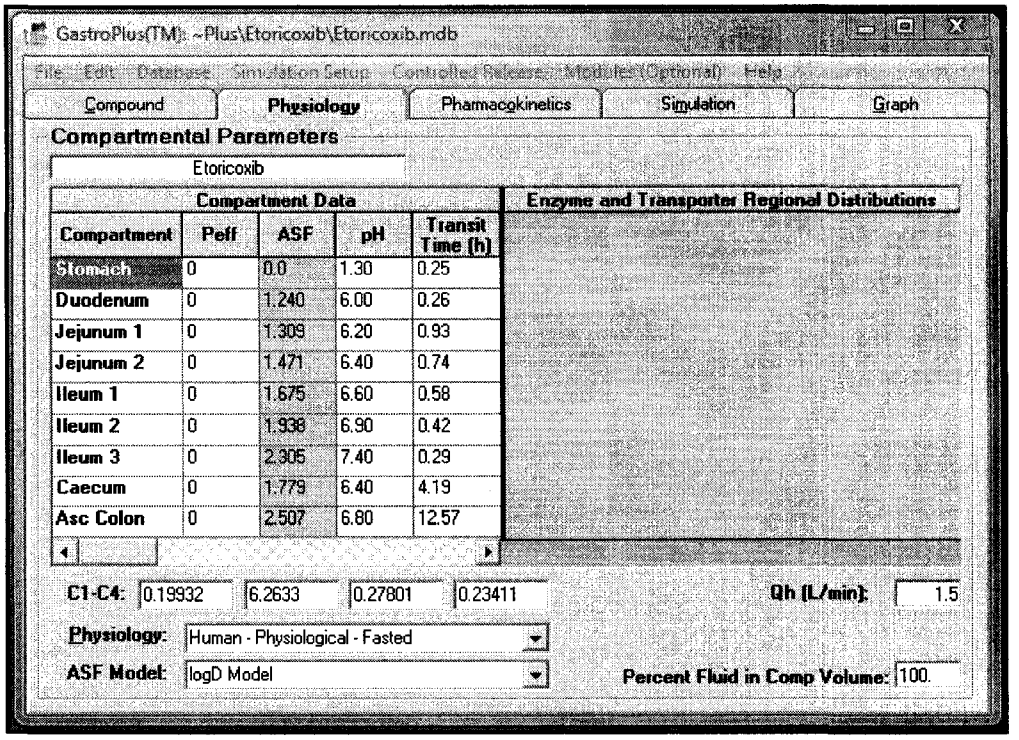

Figure-A 7 The Physiology Tab

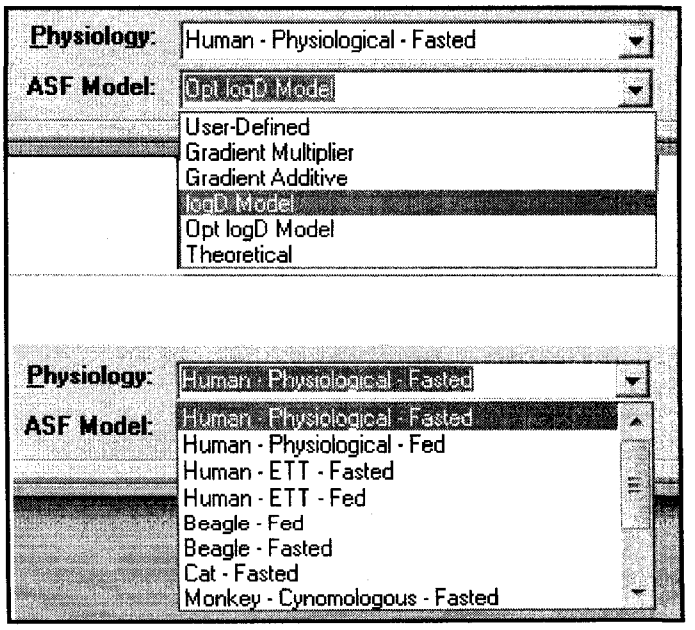

Figure-A 8 Drop-down menus for ASF and Physiology

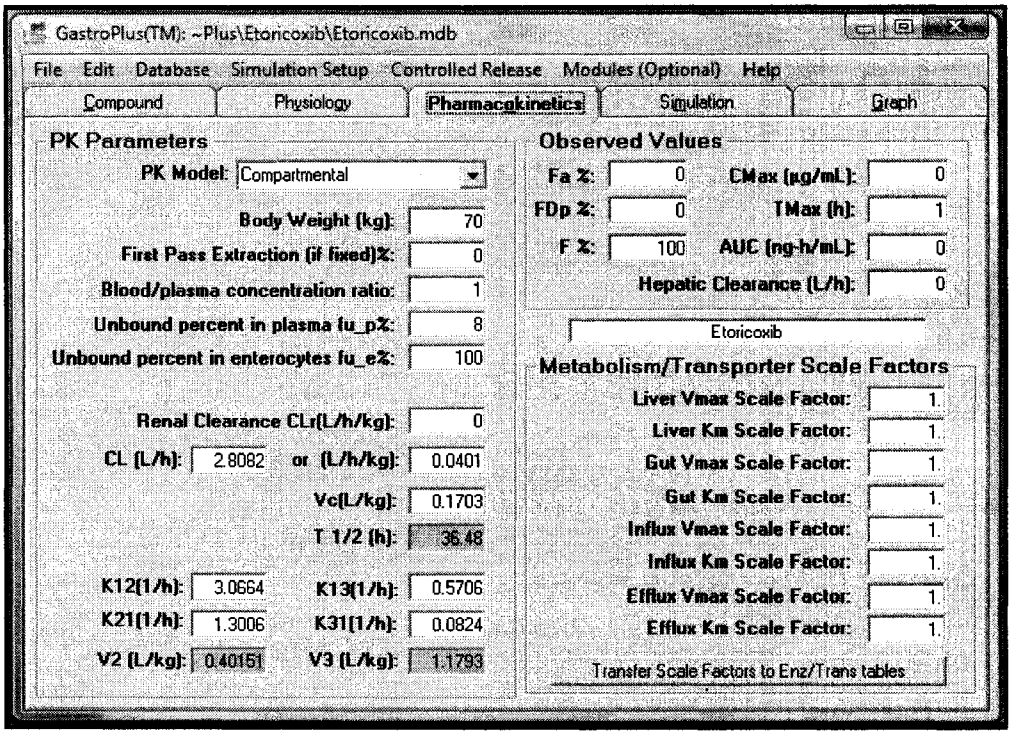

Figure-A 9 The Pharmacokinetic Tab

1.6 Performing simulations

The default single simulation mode is displayed in Figure A-10. The Graphic image of the GI tract can depict the flow of the drug material through the compartments as the simulation is running. The user needs to input the length of the simulations (example, 24 h, 48 hr or 120 h); depending on the length of time the plasma data one is simulating is collected. When the **"start"** button is clicked, simulation starts, and the button turns to **"Pause",** implying that the simulation can be paused and re-stated. When the simulation is complete, the values of the observed (from the oral data previously entered) and the simulated (or calc) fraction dose absorbed, Cmax, Tmax and AUC are displayed as in Figure A-10. To be able to see the simulated and observed profiles, the user has to go to the Graph tab and the simulated and observed plasma profiles as in Figure A-11 is displayed by clicking the **"CP-time"** button. To see the compartmental absorption pattern like in Figure A-12, click on the **"Regional Absorption"** button.

Placing the cursor on the simulated Plasma profiles displays the statistics that gives the values for the correlation coefficient between the simulated and the observed profiles (Figure A-13). This statistics provides a more in-depth analysis of how all the measured *in vitro* data were concurrently used to predict the *in vivo* profile. It is the statistics that best describes how the *in vitro* measured results correlate with the *in vivo* profile.

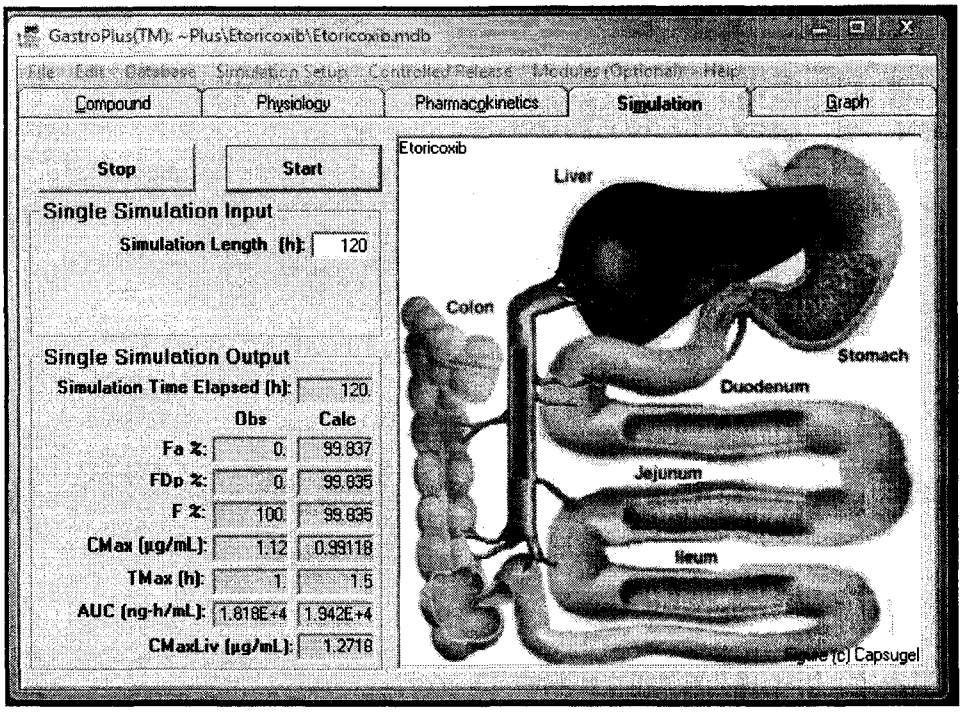

Figure-A 10 The Simulations Window

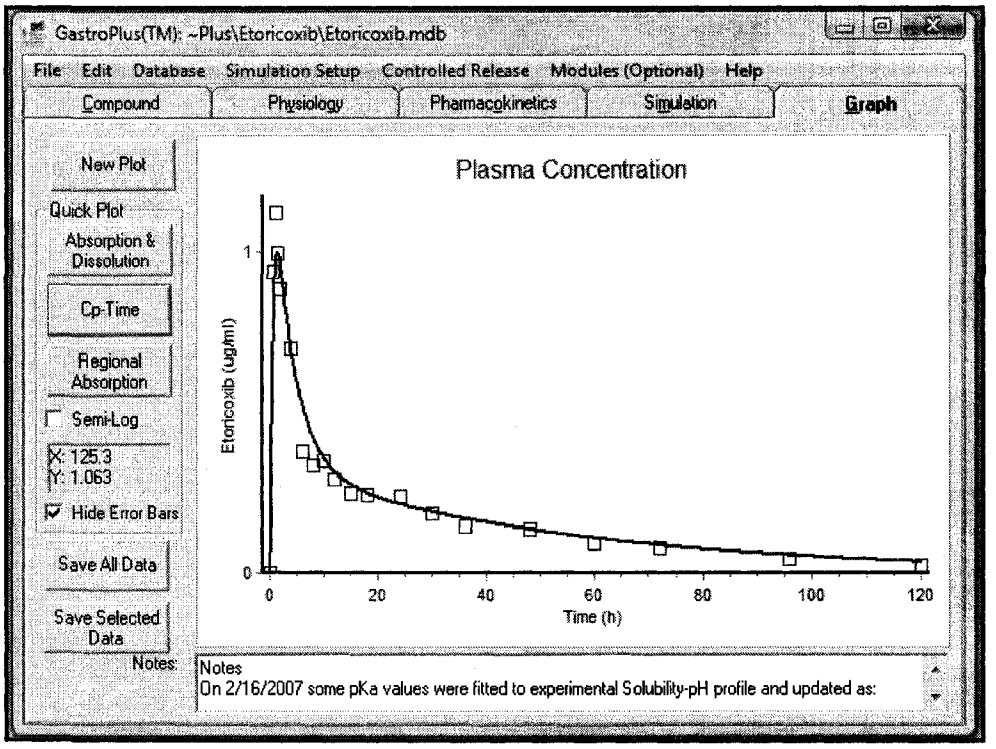

Figure-A 11 Simulations output

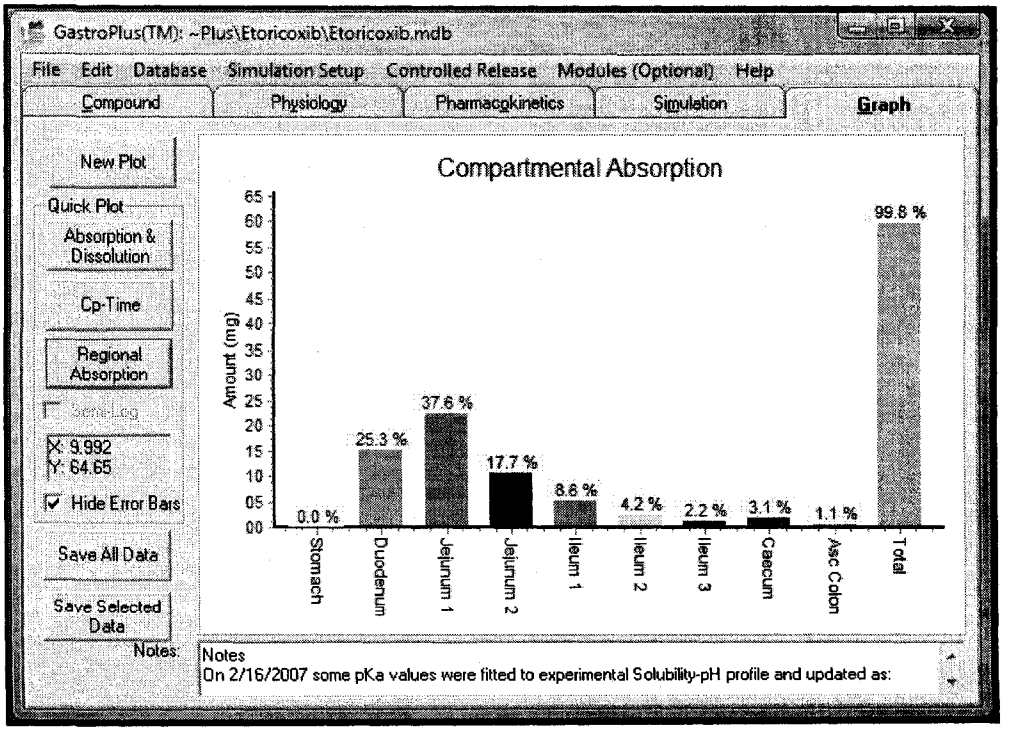

Figure-A 12 Compartmental absorption pattern screen

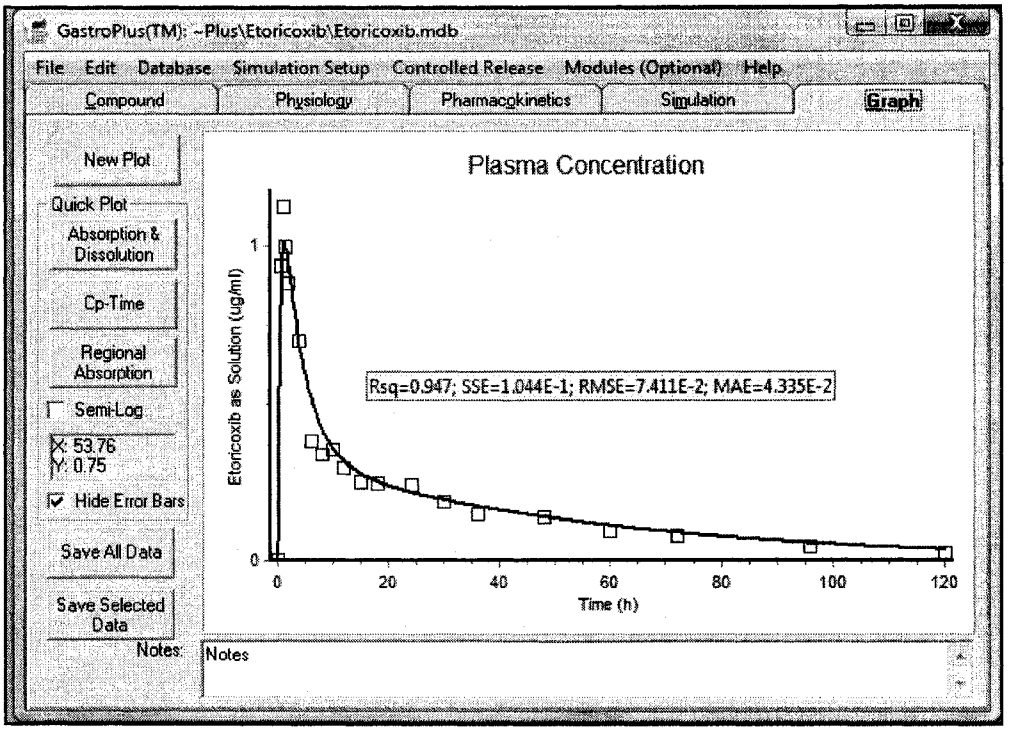

Figure-A 13 Correlations between simulated and observed plasma profiles

1.7 To create more drug records

Once all this is done, if the user has more dissolution profiles to use for simulations, the first drug record created can simply be copied and saved under a new name in the same drug database. This way, all other data that do not change, for example the pharmacokinetic rate constants, the pH solubility profile, the pKa, the oral data, the permeability, etc are left intact. All the user has to do is enter a new dissolution profile and save it in the new drug record, and they are set to simulate using a different dissolution dataset.

The above provides a simplified procedure one can follow to perform basic simulations. Details of other functionalities and other capabilities of GastroPlus™ can be found in the user manual.