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Research Paper

Dynamic Dissolution Testing To Establish *In Vitro/In Vivo* Correlations for Montelukast Sodium, a Poorly Soluble Drug

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Purpose. The objectives of the study was to develop a dissolution test method that can be used to predict the oral absorption of montelukast sodium, and to establish an *in vitro/in vivo* correlation (IVIVC) using computer simulations.
Methods. Drug solubility was measured in different media. The dissolution behaviour of montelukast sodium 10 mg film coated tablets was studied using the flow-through cell dissolution method following a dynamic pH change protocol, as well as in the USP Apparatus 2. Computer simulations were performed using GastroPlus™. Biorelevant dissolution media (BDM) prepared using bile salts and lecithin in buffers was used as the dissolution media, as well as the USP simulated intestinal fluid (SIF) pH 6.8 and blank FaSSIF pH 6.5. Dissolution tests in the USP Apparatus 2 were performed under a constant pH condition, while the pH range used in the flow through cells was pH 2.0 to 7.5. The *in vitro* data were used as input functions into GastroPlus™ to simulate the *in vivo* profiles of the drug.
Results. The solubility of montelukast sodium was low at low pH, but increased as the pH was increased. There was no significant difference in solubility in the pH range of 5.0 to 7.5 in blank buffers, but the drug solubility was higher in biorelevant media compared with the corresponding blank buffers at the same pH. Using the flow through cells, the dissolution rate was fast in simulated gastric fluid containing 0.1% SLS. The dissolution rate slowed down when the medium was changed to FaSSIF pH 6.5 and increased when the medium was changed to FaSSIF medium at pH 7.5. In the USP Apparatus 2, better dissolution was observed in FaSSIF compared with the USP buffers and blank FaSSIF with similar pH values. Dissolution was incomplete with less than 10% of the drug dissolved in the USP-SIF, and was practically non existent in blank FaSSIF pH 6.5. The *in vitro* results of the dynamic dissolution test were able to predict the clinical data from a bioavailability study best.
Conclusions. Dynamic dissolution testing using the flow through cell seems to be a powerful tool to establish *in vitro/in vivo* correlations for poorly soluble drugs as input function into GastroPlus.

KEY WORDS: ACAT; BCS; dynamic dissolution; IVIVC.

INTRODUCTION

Montelukast sodium (Singulair™), 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 (1). 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. It is a highly lipophilic drug with estimated logP of 8.79 (ADMET predictor™) and pKa of 2.7 and 5.8 (2). Its aqueous solubility is reported to be between 0.2–0.5 μg/ml in water at 25°C (2). Because montelukast contains polar and nonpolar groups at opposite ends of the molecule, the drug has amphiphilic physicochemical properties (2). The structure of montelukast sodium is shown in Fig. 1 below.

Dissolution testing is an industry standard method that is used in quality control to monitor batch to batch consistency. In Research and Development it is used to assess and estimate the *in vivo* behaviour of orally administered solid dosage forms (3). Dissolution profiles may be used to establish *in vitro/in vivo* correlation (IVIVC) (4). The development of an IVIVC for a pharmaceutical dosage form is of great interest to the pharmaceutical industry. An IVIVC can be used to request biowaivers from regulatory agencies for certain formulation or production changes within the lifecycle of a product (4, 5). This reduces the need for expensive bioequivalence testing in humans (6). They can also be used to support post-approval changes arising due to scale-up, equipment change and site change. Defining the right dissolution conditions requires an understanding of the relationship between the various physiochemical and physiological factors that have an impact on the rate and extent to which an orally administered dosage form is absorbed (7–10).

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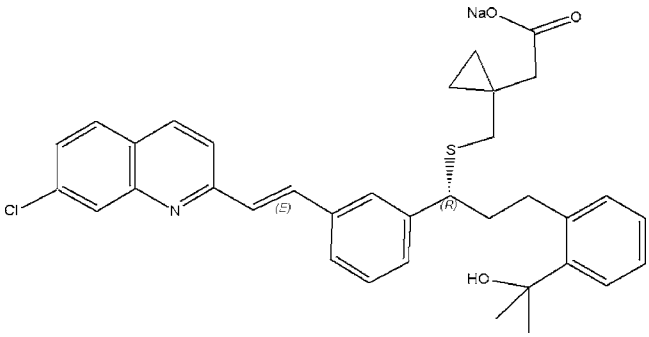

Fig. 1. Structure of montelukast sodium.

Physiologically based models for the prediction of the gastrointestinal transit and absorption of drugs in humans have received much attention recently (11–14). GastroPlus™ (version. 5.2.0; Simulations Plus Inc, Lancaster, CA, USA), is a simulation software that uses the advanced compartmental absorption and transit model (ACAT) (15). In the ACAT model the small intestine is divided into different compartments and calculates the fraction dose absorbed for each 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 (15). However, to be able to predict clinical observed plasma time curves the software requires accurate input functions which have to be estimated *in vitro*, and the *in vitro* drug release profiles must reflect the *in vivo* drug release.

In this study, the dissolution behaviour of montelukast sodium (Singulair®) 10 mg tablets was investigated using a flow through cell dissolution method following a dynamic pH change protocol, in order to establish *in vitro*/*in vivo* relationship. The USP defines *in vitro*/*in vivo* correlation 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"(16). 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.

The flow through method was chosen because of its ability to simulate the *in vivo* hydrodynamics better compared with the USP Apparatus 1 or 2, thus allowing a more close simulation of the pH gradient in the GI tract (17,18). Dissolution tests were also performed in the USP Apparatus 2 using FaSSIF and the USP-SIF. GastroPlus™ was then used to simulate and model the drug absorption process in the GI tract.

MATERIALS AND METHODS

Materials

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 (low quality, 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 μm Whatman glass microfibre filter were purchased from Life Sciences (Life Sciences Canada Inc).

Methods

Media Preparation

The USP Simulated gastric fluid (SGF) pH 1.2 (without enzymes), acetate buffer pH 4 and the USP simulated intestinal fluid (SIF) pH 6.8 (without enzymes) were prepared following the USP 27. Simulated gastric fluid with sodium lauryl sulphate (SGF-SLS) pH 2.0, without enzymes but with 0.1% SLS, was prepared as proposed by Dressman *et al.* (3).

The biorelevant media containing bile salts and lecithin were prepared following the procedure and modification outlined by Marques (19), which was adopted from the composition proposed by Galia *et al.* (20). 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 1,000 ml.

The media composition were as follows; SGF, 0.01 M HCl, consisting of 2 g/l NaCl and 0.1% *w*/*v* SLS, pH 2.0 and the BDM (FaSSIF pH 5.0, 6.5, 7.5) consisting of 3.75 mM sodium taurocholate and 0.75 mM lecithin.

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 h, filtered using a 0.45 μm Whatman glass microfibre filter (Life Sciences, Canada Inc.) and analyzed by HPLC.

Scanning Electron Microscopy (SEM)

Particle size diameter 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. The average particle diameter was estimated by measuring the projected area diameter of about 200 particles,

and the using the equation below (21) (pp 189), the size distribution was estimated.

$$d_{av} = \frac{\sum nd}{\sum n}$$

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

Dissolution Testing

Flow Through Cell 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). The cells were made following the specifications provided in the USP. The filter chambers were filled with fibre glass wool (~200 mg) and covered with a wire mesh with sieve #200 opening. A single 4 mm glass bead was placed at the bottom opening of cell and 1 g of 1 mm glass beads were added on top of it.

An open system set-up to maintain sink condition was used. A gradient pH change sequence simulating the passage of a drug through the GI tract's pH profile was used. The media used for the test were placed in a constant temperature water bath, maintained at 37±0.5°C. Media delivery was achieved using a peristaltic pump (Piper Pump, Dungey Inc. Agincourt, Ontario). During the dissolution tests, the entire fluid passing through the cells for each sampling interval was collected. The collected volume was weighed and exact volume was calculated using the previously determined density of the media.

Flow Through Dynamic Dissolution Protocol. The SGF was pumped through the cells at a flow rate of ~3.3 ml/min for 15 min. This was followed by FaSSIF pH 6.5, 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. The flow rates were estimated based on reported average fluid secretion rates in the different parts of the gastrointestinal tract per day (22).

The small intestinal transit time is about 3±1 h, for solution, pellets and single unit dosage forms (23), although an average of up to 8 h has been reported in healthy volunteers (24). In a study using indigestible telemetric capsules in healthy volunteers, the small intestinal transit time was found to be between 180 to 300 min in humans (25). The intraluminal pH in man is reported to rapidly change from highly acidic in the stomach to about pH 6 in the duodenum, then gradually increases in the proximal ileum from pH 6, to about pH 7.4–8.1 in the mid to terminal ileum, and drops to about 5.7 in the caecum (26). The time the dosage form spends in pH greater that 6.0 is variable, but a range of 2.8–8.8 h and 0.7–7.7 h at pH greater that 6.5 and 7.0 respectively, has been reported (24). Mojaverian *et al.* (25), reported the presence of a lag time of 0.8 to >2.5 h at the ileocecal junction, which acts as a valve that limits the transit into the large bowel, and the pH in this region ranges from 6.5–8.5 with a mean of 7.3. This physiological change in pH values is thought to have an impact on the solubility of weak acid drugs, which are highly soluble at high pH values. The test at pH 6.5 using the flow through dissolution method is representative of the transit through the duodenum and upper small intestine, while the test time of 150 min at pH 7.5 would represent passage through the mid and lower small intestine.

Dissolution Tests Using the USP Apparatus 2. Dissolution tests in the USP Apparatus 2 (Erweka DT 6, Germany) were performed using biorelevant media, the USP-SIF pH 6.8 and blank FaSSIF buffer pH 6.5. The media volumes used in both tests were 900 ml. The biorelevant media used was FaSSIF pH 6.5 at the recommended volumes of 500 ml (20); Marques 2004). An additional test in the FaSSIF was performed with 900 ml medium volume. Paddle speeds of 75 were used for the 500 ml and 900 ml media volumes and a 100 rpm was used for a test using the 500 ml medium volume in the USP Apparatus 2. 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 μm), the first 3 ml was discarded and the remainder was analyzed by HPLC.

HPLC Assay

The analytical column used was Inertsil Phenyl 10 cm× 3.0 mm, 5 μm (Metachem Technolgies Inc.), column temperature was maintained at 50°C and the mobile phase consisted of double distilled water with 0.2% TFA: acetonitrile with 0.2% TFA (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, using a Shimadzu LC-600 pump, with SIL-9A auto sampler (Shimadzu, Japan) and Dynamax UV detector (Dynamax Corporation, Elkhart, IN, USA). A calibration curve which was linear in the range of 1.0–35.0 μg/ml was established and used to quantify the analyte.

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 profile of the drug. The three main interfaces (tabs) used for data input are the compound, physiology and pharmacokinetic tabs. In the compound tab, the basic data pertaining to the drug's physicochemical properties such a bulk density, solubility, pKa, dose and particle radius are entered. The human effective permeability for montelukast sodium used in the simulations (9.35×10^{-4} cm^{-1}) was estimated using the ADMET Predictor™ (version 2.0, Simulations Plus Inc, Lancaster, CA, USA), as well as the LogP value. The diffusion coefficient was estimated by Gastroplus™.

The *in vitro* dissolution data were entered into Gastroplus™ using the tabulated *in vitro* dissolution 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 human LogD absorption model was used to estimate the changes in permeability as the drug travels along the GI tract. Gastroplus™ then calculates the fraction dose absorbed based on the ACAT model using drug concentration, permeability and transit times in each compartment. Values for the pharmacokinetic intercompartmental rate constants (k_1k_2, k_2k_1 etc), volume of distribution (V_d) and clearance (Cl) were estimated using the

PK Plus module 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 clinical data used in the simulations were obtained from a published report by Zhao *et al.* (38). The percent of drug lost due to first pass metabolism was calculated using data from a published pharmacokinetic study (27), and the value obtained was used in the computer simulations. First, hepatic extraction ratio was obtained using the equation by Shargel and Yu (28); and then the contribution from non-hepatic extraction was calculated, based on the bioavailability reported (27). The overall percent of first pass extraction was calculated by adding contributions from hepatic and non-hepatic extraction.

$$\text{HepaticER} = 1 - \left(\frac{\text{AUC}_{\text{oral}}/\text{Dose}_{\text{oral}}}{\text{AUC}_{\text{iv}}/\text{Dose}_{\text{iv}}} \right)$$

Where HepaticER is the hepatic extraction ratio, AUC_{oral} is the area under the plasma concentration-time curve obtained after administering the oral dose, $\text{Dose}_{\text{oral}}$ and AUC_{iv} is the area under the plasma concentration-time curve obtained after administering the intravenous dose, Dose_{iv}.

Statistical Analysis

The dissolution profiles were compared using the difference factor (f_1) and similarity factor (f_2) (29) using the following equations:

$$f_1 = \frac{\sum_{i=1}^{n} |R_i - T_i|}{\sum_{i=1}^{n} R_i} \times 100 \text{ and}$$

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

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.

Linear regression analysis to compare the simulated and observed plasma profiles were automatically generated using Gastro plus™. Values displayed included the regression coefficient (r^2), the sums of square error (SSE), the root mean square error (RMSE and the mean absolute error of prediction (MAE). The percent prediction error (PE) was estimated using the equation (30):

$$\%\text{PE} = \frac{\text{observed} - \text{predicted}}{\text{observed}} \times 100$$

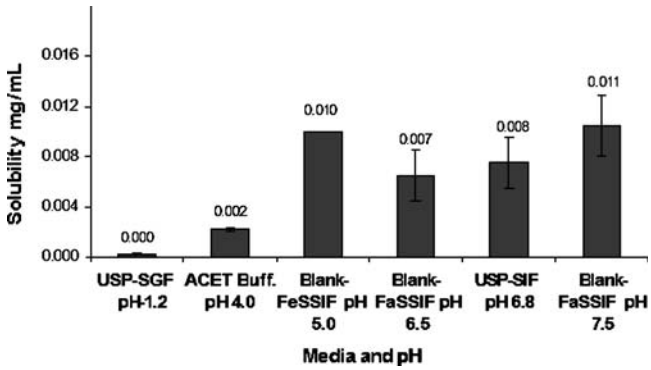

Fig. 2. Solubility of montelukast sodium in blank biorelevant media and USP-buffers.

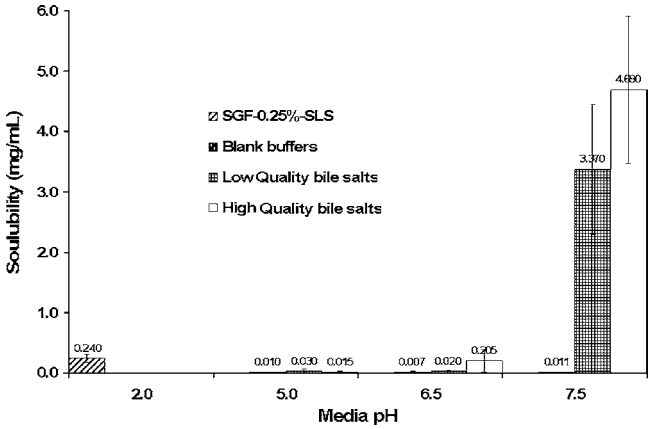

Fig. 3. Solubility of montelukast sodium in SGF with SLS and biorelevant media containing high and low quality bile salts and lecithin.

RESULTS

Solubility in Different Media

Figures 2 and 3 show the solubility results of montelukast sodium at different pH values. The solubility in the USP-simulated gastric fluid pH 1.2, ~0.18 μg/ml or (0.00018 mg/ml). In the presence of sodium lauryl sulphate at pH 2.0, its solubility increases by more than 1,000 fold to 0.24 mg/ml. Between pH 4.0 and 5.0 the solubility increases fivefold and there is no significant difference in solubility in the pH range of 5 to 7.5 in blank buffers (Fig. 3). The solubility in biorelevant media is higher than in the corresponding blank buffers at the same pH. The highest solubility was obtained in biorelevant media at pH 7.5. The drug has a higher solubility in media prepared using high quality bile salts (4.69 mg/ml) compared with media prepared using crude bile salts (3.37 mg/ml; Fig. 3).

Particle Size Evaluation

Table I shows the particle size distribution of montelukast sodium. About 87% of the drug particles are below 20 micrometer in diameter. The mean particle size estimated using the microscopic method is 11.56±9.7 μm. The particle size distribution (as radius) was used in Gastroplus™ as a support file during simulations.

Dissolution Tests

Figure 4 shows the mean dissolution profile of montelukast sodium (10 mg tablets) using the dynamic pH change flow through protocol and in the USP Apparatus 2 using various media at constant pH. In the flow through cells the dissolution rate changes as the pH of the medium is changed. The dissolution rate is relatively fast in SGF—0.1% SLS at

Table I. Montelukast Sodium Particle Size Distribution

Particle diameter (μm)	Percentage per group (%)
<10	59.0
10–20	28.5
20–30	7.0
30–40	3.5
>40	2

Table II. Table of f_1 and f_2 Test Results

	f_1	f_2	Status
FaSSIF-900 ml 75 rpm	5.0	54.6	Similar
FaSSIF-500 ml 75 rpm	6.9	48.0	Not similar
USP-SIF	292.5	12.1	Not similar

The profile in the FaSSIF-500 ml–100 rpm was used as reference

pH 2.0, it slows down at pH 6.5 and increases again when pH 7.5 was used. The final change to pH 5.0 seems not to impact the dissolution which might be due to the small amount of un-dissolved drug left over at this time point.

In the USP Apparatus 2 using various media, better dissolution was observed in FaSSIF compared with the USP-SIF and blank FaSSIF. At the end of the dissolution test (240 min), 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 less than 10% of the drug is dissolved and dissolution in blank FaSSIF pH 6.5 was practically nonexistent.

Table II shows the similarity and difference factor test results, when the profiles from dissolution tests in the USP Apparatus 2 are compared. The dissolution profile in FaSSIF-500 ml–100 rpm was used as reference, because it showed a better fit in simulations compared with FaSSIF-500 ml–75 rpm.

Computer Simulations

Simulations Using Dissolution Data

Figure 5 shows the simulated plasma profiles using the flow through cell dissolution data as input function into GastroPlus with and without first pass extraction correction, and also the simulated profiles using data from the USP Apparatus 2 dissolution (first pass extraction applied). The simulated profile using the flow through dissolution data with first-pass extraction correction appears to match the *in vivo* profile well, compared with simulated profile without first pass extraction correction. The simulated profiles using the dissolution data from FaSSIF in the USP Apparatus 2 and at different agitation rate do not predict the observed *in vivo* profile well. The simulated profile using the USP-SIF data (where less than 10% of the drug is dissolved), is close to baseline.

Linear regression analysis results are shown in Table III. Simulations using dissolution profile from the flow through cell provided the best IVIVC (r^2=0.979), followed by 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 and FaSSIF-500 ml–75 rpm result into weak correlations, with r^2=0.756 and 0.683 respectively. The USP-SIF has almost no correlation at all. The SSE, RMSE and MAE increases as the correlation gets weaker.

The percent prediction error (Table III) shows that the flow through cell and FaSSIF-500 ml–100 rpm dissolution data under-predicted AUC by 1% and 1.7% respectively, while they over-predicted the C_{max} by 18.2%, 11.6% respectively. The dissolution data from FaSSIF-900 ml under-predicts the 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 USP-SIF has the highest percent prediction error, indicating that it is not a suitable medium for dissolution testing of montelukast sodium when attempting to establish an IVIVC.

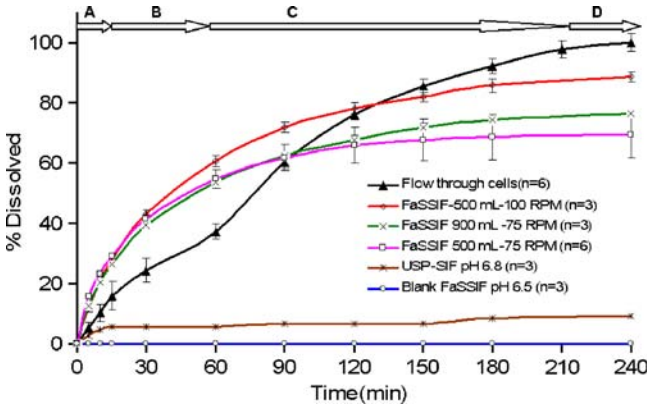

Fig. 4 Dissolution profiles of montelukast sodium 10 mg film coated tables in the flow through cells following dynamic pH change protocol and in the USP Apparatus 2 using different media and agitation rates at constant pH values. The *lines* indicate the time point at which the media was changed to the respective pH values in the flow through cell dissolution profile only. The areas labelled *A*, *B*, *C* and *D* indicates the respective pH values of the medium between each of the time points. (*A* pH 2.0; *B* pH 6.5; *C* pH 7.5 and *D* pH 5.0).

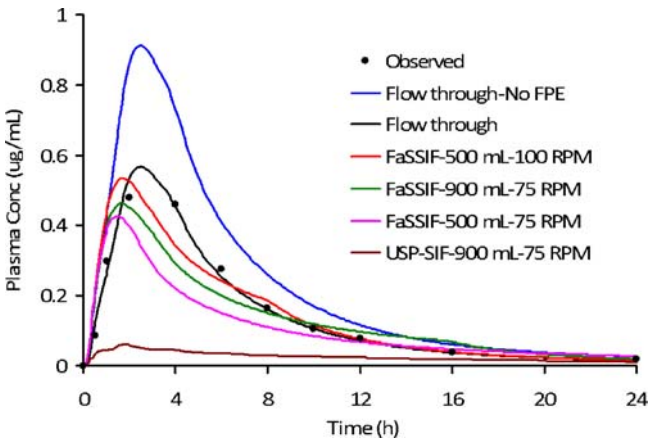

Fig. 5. Comparison of simulated profiles from the flow through and from the USP Apparatus 2 using different media. Note: (No FPE= profile from the flow through dissolution with No first pass extraction applied). All the rest of the simulated profiles had a 38% first pass extraction applied.

Table III. Linear Regression Analysis (No FPE=No First-Pass Extraction Applied)

Medium/Method	Power of prediction Values			
	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
Flow through—No FPE	0.758	0.277	0.166	0.124
USP-SIF	0.359	0.526	0.229	0.171

Discussions

Solubility in Different Media

The higher solubility of montelukast sodium in SGF-SLS compared with USP-SGF without enzymes is likely due to the better solubilizing effect of the surfactant (SLS).

Similarly, the high solubility of montelukast sodium in FaSSIF 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 due to the high lipophilicity of the drug. This is supported by the high LogP value (8.79) obtained for montelukast sodium using the ADMET Predictor™. A high pH alone does not appear to increase the solubility of the drug. This is also in accordance with reports by Jinno *et al.* (31) where they found that the solubility of Piroxicam, an ionizable weakly acidic drug increased with an increase in the pH and the surfactant concentration. They attributed this to the combined effect of the surfactant and the pH.

The highest solubility of montelukast sodium was observed in biorelevant media at pH 7.5. However there was a difference in solubility between media prepared using high quality bile salts (4.69 mg/ml) compared with media prepared using crude bile salts (3.37 mg/ml) at the same pH. This might be due to purity of the bile salts and lecithin used. Mithani *et al.* (32), reported an enhanced drug solubility in high purity sodium taurocholate solution, for lipophilic drugs whose logP values are greater than 2.5. Crison *et al.* reported similar results for the solubility of cabamezepine and the purity of the surfactant (SLS) used to prepare the media (33).

Other reports from Wei and Löbenberg (13) however show that glyburide had better solubility in low quality FaSSIF compared with high quality FaSSIF. Hammad and Müller (34) in their report concluded that enhancement of solubility of drugs by the different grades of bile salts and lecithin depends on the chemical nature of the drug, when they compared the solubility of steroidal drugs and benzodiazepines in different grades and composition of bile salts and lecithin.

Although the general trend indicates that the solubility of montelukast sodium is pH dependent, the results show that the presence of surfactants has a significant impact on the solubility of montelukast sodium, within the pH range at which these experiments were performed. Based on the entire pH range of 1.2 to 7.5, montelukast sodium has to be considered as a poorly soluble drug and fits best into BCS class 2 or 4. Montelukast sodium is reported to have two pKas (pka_1=2.7 and pKa_2=5.8), thus causing the drug to have amphiphilic behaviour (2). The value of pKa_2 is within the biological pH range in the small intestine, therefore it is expected that its dissolution behaviour *in vivo* varies as the drug moves down the GI-tract, with faster dissolution taking place further down in the gut where the pH is higher.

Dissolution Tests

In the USP Apparatus 2, a higher percent drug dissolved in FaSSIF-500 ml at 100 rpm (88.9%) compared, with 69.4% when 75 rpm indicates that the agitation speed has an impact on the rate and extent of dissolution in FaSSIF at pH 6.5. The 100 rpm appears to provide better hydrodynamic conditions compared with 75 rpm. This is in accordance with the report by Wu *et al.* where they found that the dissolution rates of theophylline, a highly soluble drug and naproxen, a poorly soluble drug increased with the increase in the paddle speed used in the dissolution testing (35). However, incomplete dissolution at both speeds might be due to a lack of sink conditions. At 0.02 mg/ml solubility in FaSSIF pH 6.5, a 10 mg dose in 500 ml, is already at saturation point. It would require a minimum of 1.5 l of FaSSIF pH 6.5 for sink conditions to prevail. The *f* test for similarity show that the dissolution profile in FaSSIF-900 ml–75 rpm is similar to FaSSIF-500 ml–100 rpm (f_2=54.6), indicating that complete dissolution can be achieved with a higher volume of dissolution media, while using a lower agitation rate. The dissolution profiles in the USP-SIF failed to match the profiles in biorelevant media. This is likely due to the poor wetting of the drug particles by the buffer system as a result of the high lipophilicity of the drug.

Table IV. Percent Prediction Error (PE) Statistics (No FPE=No First-Pass Extraction Applied)

Media	AUC (ug.h/ml)	Cmax (ug/ml)	AUC %PE	C_{max} %PE
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
Flow through-No FPE	5.67	0.913	−59.6	−90.3

Observed values: AUC=3.552 μg.h/ml; C_{max}=0.4796 μg/ml

Perng *et al.* (36) reported a reasonable correlation between dissolution data from the flow through cell following a gradient pH change sequence and bioavailability of SB-247083, which enabled their group to select the most appropriate formulation to progress to clinical trials. Sunesen *et al.* (37) reported establishing *in vitro*/*in vivo* correlations for a poorly soluble drug, danazol using the flow through dissolution testing and biorelevant dissolution media. In their study however they used a single flow rate and single pH medium at a time, with a range of different flow rates from as low as 8 to 32 ml/min (37).

Wei *et al.* (13) had shown for glyburide that a dynamic pH change protocol can exhibit significant differences in the drug release profile compared to single pH conditions in the same apparatus. They used in their study a USP paddle apparatus rather than a flow through cell and changed the pH overtime. In the present study, the dynamic pH change protocol was applied to the flow trough cell. Since this is one of the first studies which uses such a protocol together with computer simulations, more data are needed to optimize and validate a universal dynamic pH change protocol for the flow through apparatus.

In the flow through cell, a higher dissolution rate in SGF-SLS pH 2.0 was observed compared with FaSSIF pH 6.5, this most likely due to the increased wetting and solubilization of the drug by the SLS present in the medium, even though the flow rate is lower in SGF compared with FaSSIF. This could also be due to the fact that the drug showed a high solubility in SGF medium containing SLS compared with hydrochloric acid alone of the same pH and FaSSIF of pH 6.5. The increased dissolution rate at pH 7.5 can be attributed 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. Galia *et al.* (20) reported an increase in dissolution of poorly soluble drugs in the presence of bile salts and lecithin. The observed dissolution profiles under dynamic pH changes suggest that *in vivo*, the dissolution rate of montelukast sodium might also change as the drug particles travel along the gastrointestinal tract. Complete dissolution can be expected to take place in the distal part of the small intestine.

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.* (38). Different values for the oral bioavailability of montelukast sodium have been reported in literature. In elderly and young healthy subjects bioavailability (BA) values of 61% and 62% were reported (38), while Cheng *et al.* (27) reported bioavailability values ranging between 58–66% in healthy male and female subjects.

The reason for the low bioavailability of Montelukast sodium is due to extensive hepatic metabolism, with the major pathway for excretion being through the bile (39,40). The major enzymes responsible for its 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.

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 from the flow through cell dissolution, using a 38% first pass extraction correction and without correction. Using this value lowered the bioavailability of the simulations to literature values and the observed and predicted values came close to each other. This comparison showed that in order to predict the *in vivo* behaviour of montelukast sodium from dissolution data, first pass extraction needs to be taken into consideration. All other simulations were therefore performed using a 38% first pass extraction correction.

The Simulations results showed that the drug is completely absorbed and literature data indicate that the low bioavailability is due to first pass extraction therefore, montelukast sodium would best be classified as a BCS class 2 drug. The flow through dissolution data established the best IVIVC as can be seen from the value of the correlation coefficient and the lowest sums of square error of prediction (SSE). The prediction error statistics for AUC is low (Table IV), well below the 10% as per the FDA requirements for the flow-through cell, FaSSIF-500 ml–100 rpm and FaSSIF-900 ml–75 rpm. However, for the $C_{max,}$ only the FaSSIF-500 ml–100 rpm and FaSSIF-900 ml–75 rpm appear to satisfy the FDA requirements. The Flow-through profile PE appears to be a little too high (18.2%). This could be due to the sampling protocol used in the clinical study, such that the actual C_{max}, which could have occurred between the 2 and 4 h sampling time points, was missed. Nevertheless, the flow through dissolution profile defined the entire plasma time curve better compared with all the rest of the dissolution profiles.

Conclusion

This study showed that the flow-through dissolution test following dynamic pH change protocols are able to mimic the environmental changes that an orally administered drug can encounter in the GI tract. Simulations using the flow through dissolution data were able define the entire *in vivo* profile of montelukast sodium and an *in vitro*/*in vivo* correlation was established. Its bioavailability appears to be dissolution rate controlled and not absorption controlled. Computer simulations using dissolution data obtained following dynamic pH change protocols appears to be a promising powerful tool for establishing *in vitro*/*in vivo* correlations for drugs whose absorption is dissolution rate controlled. The flow-through dissolution testing using the open system and following a dynamic pH change sequence is a promising bio-relevant dissolution test method.

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