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Dissolution profiles of BCS class II drugs generated by the gastrointestinal simulator alpha has an edge over the compendial USP II method

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ABSTRACT

The poor water solubility of orally administered drugs leads to low dissolution in the GI tract, resulting to low oral bioavailability. Traditionally, in vitro dissolution testing using the compendial dissolution apparatuses I and II has been the gold-standard method for evaluating drug dissolution and assuring drug quality. However, these methods don't accurately represent the complex physiologies of the GI tract, making it difficult to predict in vivo behavior of these drugs. In this study, the in vivo predictive method, gastrointestinal simulator alpha (GIS-α), was used to study the dissolution profiles of commercially available BCS Class II drugs, danazol, fenofibrate, celecoxib, and ritonavir. This biorelevant transfer method utilizes multiple compartments alongside peristaltic pumps, to effectively model the transfer of material in the GI tract. In all cases, the GIS-α with biorelevant buffers gave superior dissolution profiles. In silico modeling using GastroPlus™ yielded better prediction when utilizing the results from the GIS-α as input compared to the dissolution profiles obtained from the USP II apparatus. This gives the GIS-α an edge over compendial methods in generating drug dissolution profiles and is especially useful in the early stages of drug and formulation development. This information gives insight into the dissolution behavior and potential absorption patterns of these drugs which can be crucial for formulation development, as it allows for the optimization of drug delivery systems to enhance solubility, dissolution, and ultimately, bioavailability.

1. Introduction

The oral route for drug delivery is the most desired route of drug administration because it is simple, non-invasive, does not require any specific sterile conditions, and has the highest patient compliance.[1] The challenge is that the drug must exhibit a sufficient bioavailability profile for pharmacological effect, and the most prominent factor that dictates this is the drug's solubility and permeability.

The Biopharmaceutics Classification System (BCS) classifies oral drugs according to their solubility and permeability and is divided into four classes: Class I (high solubility and permeability), Class II (low solubility and high permeability), Class III (high solubility and low permeability), and Class IV (low solubility and permeability).[2] Approximately 40 % of orally administered drugs that are available in the market fall into the low solubility category (BCS class II and IV). Moreover, recent reports indicate that up to 70 % of drugs in the current drug discovery pipeline belong to BCS class II, while another 20 % belong to BCS class IV.[1,3] This poses a significant challenge for researchers to optimize oral formulation and to assure the bioavailability of these drugs for the desired therapeutic outcomes. For this challenge, appropriate dissolution tests are crucial to predict in vivo behavior of oral dosage form in the GI tract and help researchers to optimize its formulation for sufficient bioavailability.

Different tools are available for researchers and pharmaceutical experts to investigate the dissolution and absorption of drug formulations. The United States Pharmacopeia (USP) dissolution apparatuses, USP I (basket) and USP II (paddle), are widely considered as the gold-standard methods for in vitro dissolution testing.[4] It uses one chamber filled with the buffer of choice that is stirred using a rotating basket or a paddle. However, it is important to note that these methods do not represent the complexities of GI physiologies such as variable pH, gastric emptying, intestinal fluid volume, buffer capacity, and hydrodynamics.[4,5] This limitation could lead to inaccurate in vitro-in vivo correlations (IVIVC) especially for poorly soluble drugs. In contrast, the multi-compartment transfer dissolution system, like the gastrointestinal simulator alpha (GIS-α), could come much closer to in vivo conditions. GIS-α consists of four chambers representing the stomach, duodenum, jejunum, and ileum and can also be extended to model the colon. It can

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be programmed to simulate a specific gastric emptying time and small intestinal transit time while also displaying the similar physiological pH change and movements in the GI tract.[6,7] In the past, the GIS-α has been demonstrated to be capable of predicting the in vivo dissolution of dipyridamole and ketoconazole, both BCS class II drugs.[6] These unique capabilities make the GIS-α one of the most practical commercially available in vivo predictive dissolution apparatuses.

The aim of this project is to test out the capabilities of the GIS-α in predicting the in vivo behavior of four commercially available drugs belonging to BCS Class II, namely celecoxib, danazol, fenofibrate, and ritonavir. In the BCS system, class II can be further divided into three: (1) IIa for weak acids, (2) IIb for weak bases, and (3) IIc for neutral drugs.[2,8] Fenofibrate and danazol are both neutral and it is expected that their dissolution will be unaffected by pH changes and would depend solely on the physicochemical properties of both drugs and the overall transit time in the GI tract. On the other hand, ritonavir is a weak base while celecoxib is a weak acid, and both are expected to have pH-dependent solubilities.[9,10] The dissolution profiles of those 4 drugs were generated using the USP II apparatus and GIS-α with 2 different buffers and compared. Overall, the results showed that the use of biorelevant media in the GIS-α gives better results than using conventional buffers and the USP II. Although promising, the method needs to be further optimized and certain checks need to be placed before it can be standardized for routine use involving low-solubility compounds.

2. Materials and methods

2.1. Materials

Celecoxib capsule (400 mg) and ritonavir tablet (100 mg) were obtained from Aurobindo Pharma USA, Inc. (East Windsor, NJ). Fenofibrate (134 mg) and danazol (100 mg) capsules were obtained from Glenmark Pharmaceuticals USA Inc. (Mahwah, NJ) and Teva Pharmaceuticals USA, Inc. (Fairfield, NJ), respectively. Acetonitrile, trifluoroacetic acid, phosphoric acid, sodium dihydrogen phosphate monohydrate, sodium chloride, and methanol were purchased from Fisher Scientific Inc. (Pittsburgh, PA). Fasted state simulated gastric fluid (FaSSGF), fasted state simulated intestinal fluid (FaSSIF), and 3F powder were purchased from Biorelevant.com (London, UK). All chemicals were of analytical grade or HPLC grade, unless otherwise specified.

2.2. Preparation of dissolution media

Simulated gastric fluid (SGF) was prepared by making a 0.01 M HCl solution at pH 1.6. The two times concentrated simulated intestinal fluid (SIF) consisted of 100 mM Na_2HPO_4 at pH 6.8 with 30 mM NaCl. The biorelevant media, FaSSGF and FaSSIF, were prepared by dissolving the appropriate amount of 3F powder in the respective buffer concentrates all in accordance with manufacturer's specifications (Biorelevant; London, UK).

2.3. Dissolution using the USP II (paddle) apparatus

The USP II (Hanson Vision8 Elite, Chatsworth, CA) is a common analytical tool used to evaluate drug release profiles in pharmaceutical research. It consists of dissolution vessels equipped with rotating paddles as the stirring element.[11] This system is equipped with standard sized paddles, an autosampler (Vision AutoPlus, Chatsworth, CA), and a filtration system (Vision AutoFilter, Chatsworth, CA), fitted with automation-compatible type APFB 1 μm filters (MilliporeSigma, Darmstadt, Germany) was used. The dissolution chambers were filled with 500 mL of simulated intestinal fluid (SIF, 50 mM phosphate buffer pH 6.8) and the temperature was maintained at 37° C using a water bath. The paddle speed was set at 75 rpm. The drug formulations were placed in a spiral sinker and were then dropped in the dissolution chamber and 1 mL samples were taken out at 5, 10, 20, 30, 60, and 90 mins. The collected samples were then mixed with an equal volume of methanol and the concentration was determined using HPLC analysis.

2.4. Dissolution using the GIS-α (Conventional)

The GIS-α dissolution apparatus, shown in Fig. 1, consists of different compartments that can be assigned as the different parts of the GI tract. In this transfer dissolution method, the software can be programmed to have specific transfer rates and rotational speeds in each chamber. All pumps were calibrated prior to use. In this study, three compartments representing the stomach, duodenum, and jejunum were used. The gastric chamber was filled with 50 mL of SGF and 250 mL of water as the dose volume, representing the standard clinical protocol of administering an 8 oz glass of water along with the dose.[12] The duodenal chamber was filled with 50 mL of SIF and the volume was kept constant throughout the experiment. The jejunal chamber simply collected the output coming from the duodenal chamber. During the experiment, SGF and 2 × SIF were kept at separate compartments and pumped into the gastric and duodenal chambers, respectively, at a rate of 1 mL/min, to maintain their pH environment. The solution transfer rate was programmed with the first-order like kinetics as reported previously with the gastric half-emptying time set to 8 min.[6] This gastric half emptying time is in accordance to reported values in fasted humans for liquids, which is from 8 to 15 mins.[13–15] The paddle speed was set at 100 rpm in the stomach but was kept at 50 rpm in the duodenum and jejunum. The whole set up was kept at 37° C by using a water bath. To initiate the experiment, the dosage form was dropped into the stomach compartment. A spiral sinker was used for dosage forms in capsule form to prevent them from floating. A 750 μL sample was taken from all three chambers at 5, 10, 20, 30, 60, and 90 min. The samples were then spun at 16400 × g for 1 min. Five hundred μL of the supernatant was taken and mixed with 500 μL of methanol to prevent precipitation. The samples were then analyzed using HPLC.

2.5. Dissolution using the GIS-α (Biorelevant)

All program settings, transfer volumes, sampling time and handling were kept the same as the conventional dissolution experiment except that the biorelevant dissolution uses the biorelevant media, FaSSGF and FaSSIF, to mimic in vivo conditions closer. Fifty mL of FaSSGF, pH 1.6, and 250 mL of water as dose volume was placed in the gastric chamber. The duodenal chamber was filled with 50 mL of FaSSIF, pH 6.8. During the experiment, FaSSGF and 2 × FaSSIF were pumped into the gastric and duodenal chambers, respectively, at a rate of 1 mL/min. Samples were collected at the specified time points, centrifuged to remove undissolved particles, diluted with the same volume of methanol, and then finally analyzed using HPLC.

2.6. HPLC analysis

A Waters HPLC system (Waters Corporation, Milford, MA) composed of Alliance 2696 and Waters 2487 UV detector controlled by Empower 3® software was used to analyze all samples. HPLC grade water with 0.1 % phosphoric acid (Solvent A) and HPLC grade acetonitrile with 0.1 % trifluoroacetic acid (Solvent B) was used as the mobile phase. Ten microliters of sample were injected and resolved in an Atlantis T3 (Waters Corporation, Milford, MA) 5 μm, 4.6 × 50 mm C18 analytical column. Gradient elution was employed from 40 % to 90 % solvent B for a period of 2 min then constant at 90 % solvent B for another 2 min before going back down to 40 % at a constant flow rate of 1 mL/min. The UV detector was set at 254 nm for all compounds. Standard curves for all drugs were also generated and used to quantify the area under the peaks for each compound. Each concentration was obtained from the area under the peak. The dissolved amount was determined based on the concentration and the volume in the vessel at the specific time. The percent drug

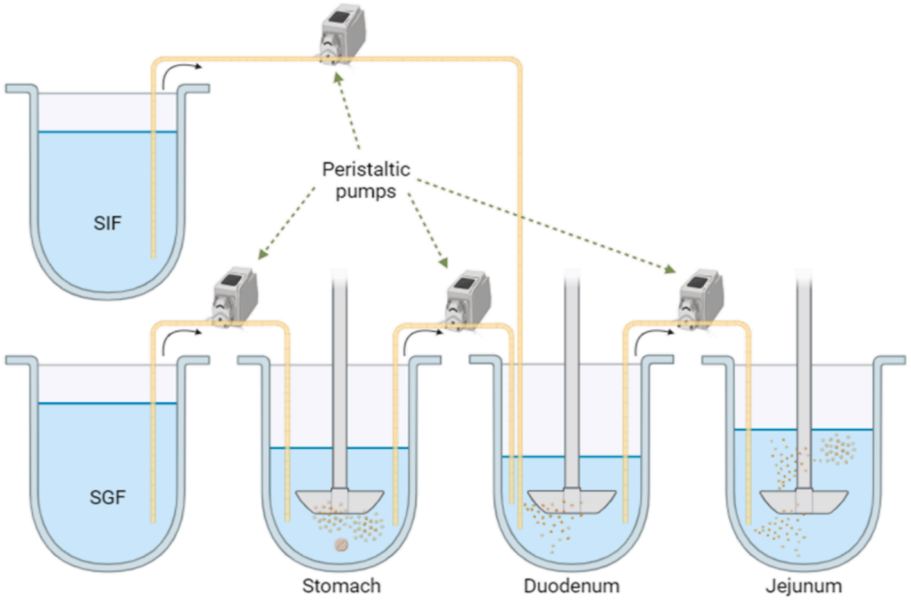

Fig. 1. Schematic diagram of the GIS-α showing three compartments representing the stomach, duodenum, and jejunum. It can be further extended with two additional compartments representing the ileum and the colon.

dissolved was calculated based on the dissolved amount divided by the dose then multiplied by 100.

2.7. Modeling using GastroPlus™

GastroPlus™ is a mechanistically based simulation software package that simulates drug absorption through various routes of administration. It is one of the most commonly used physiologically-based pharmacokinetic (PBPK) modeling software in the pharmaceutical industry and helps in evaluating in vivo performance of drug products and formulations. However, not every single variable in the human GI tract can be accounted for due to lack of appropriate physiological data. Nonetheless, countless studies involving the use of GastroPlus in PBPK modeling is present in the literature and has been clinically verified.[16] The computational software, GastroPlus™ 9.8.3 (SimulationPlus, Inc., Lancaster, CA), was ran using a Lenovo ThinkPad computer with Intel Core i5 processors. With this software, one can input in vitro dissolution profiles alongside known physicochemical and pharmacokinetic (PK) parameters in the literature. The parameters used are summarized in Table 1, and the software will calculate in vivo performance and PK parameters for each drug. A standard physiological condition model within the software was used: Human Physiological-Fasted alongside the Opt LogD Model SA/V 6.1. Results are then compared to clinical data available in the literature.

3. Results and discussion

The initial step in oral drug absorption is the release of the drug from the formulation matrix, either by tablet disintegration or by capsule rupture, followed by dissolution which is affected by several factors such as the physicochemical properties of the drug, the formulation, excipients, particle size, pH, and whether food is present or not.[23,24] This process often starts in the gastric environment. Using three compartments of the GIS-α, the dissolution profiles of four model drugs were obtained in the gastric, duodenal, and jejunal chambers with two different buffer systems. The first one is by using the simple buffers, SGF and SIF. The second setup involves FaSSGF and FaSSIF buffer to be closer to biorelevant conditions.[25] Bile micelles present in the biorelevant buffers are also known to affect solubility, and dissolution rate of low-solubility compounds.[26] The change in pH in the gastric and duodenal compartments was also monitored using a pH probe. The four model drugs studied here are shown in Fig. 2.

Table 1
Physicochemical and pharmacological properties of each drug for GastroPlus™ simulation.

	Danazol	Fenofibrate	Celecoxib	Ritonavir
MW, g/mol	337.45	360.8	381.38	720.96
Dose, mg	100 (capsule)	134 (capsule)	400 (capsule)	100 (tablet)
Dose Number	400*	1,914*	400*	181.8*
Dose Volume, mL	250	250	250	250
Solubility, mg/mL	1.0×10^{-3} [a]	2.8×10^{-4}[c]	3.8×10^{-3} [e]	3.85×10^{-2}[h]
log*P*	4.53[b]	5.3[d]	3.9[e]	3.9[h]
pK_a	–	–	10.7[f]	1.8[h]
Mean precipitation time, s	900*	900*	900*	900*
Human P_{eff}, $\times 10^{-4}$ cm^2/s	2.76[a]	2.83[d]	3.07[e]	2.76[i]
Body weight, kg	70	70	70	70
Blood:plasma ratio	0.91*	0.7*	0.89[g]	0.64*
Fraction unbound in plasma	5.83*	0.1*	3[g]	0.015[i]
V_c, l/kg	1.06*	0.4[d]	2.40[g]	0.410[i]
CL, (L/hr/kg)	1*	0.02[d]	0.379[g]	–

* Predicted values in GastroPlusTM using ADMET Predictor v10.4.0.0.
[a][7].
[b][17].
[c][18].
[d][8].
[e][19].
[f][10].
[g][20].
[h][21].
[i][22].

3.1. Monitoring the pH in the stomach and duodenum

The added capability of the GIS-α to monitor pH in each chamber allows for examination of any pH change as the dissolution experiment goes on. The pH in the stomach and the duodenum were monitored and results are shown in Fig. 3. In the stomach chamber, a higher pH value at

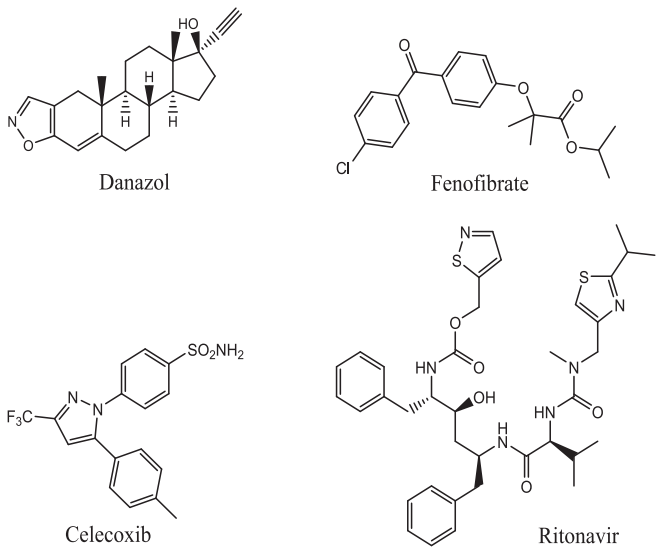

Fig. 2. Chemical structure of the BCS Class II drugs used in this study.

the beginning is observed because of dilution effect from the 250 mL water dose. This value gradually decreases as concentrated gastric fluid is re-introduced and the total volume in the stomach goes down. In the duodenal chamber, the pH dips down at the early timepoints due to the transfer of acidic solution from the gastric chamber. This then recovers because of the constant secretion of 2 × concentrated intestinal fluid. This is more evident in the biorelevant buffer, FaSSIF. The SIF buffer was designed to keep the pH constant all the time due to its high buffer capacity and its buffer capacity may not be physiologically relevant. This type of media is useful for evaluating the rate of drug dissolution with respect to gastric emptying time and is often used in compendial dissolution apparatuses in evaluating BCS class I and III compounds.[27] On the other hand, the FaSSGF and FaSSIF buffers have additional physiological layers by modeling the fasted state, the presence of physiological surfactants (bile salts and lecithin), and biorelevant buffer capacity to better simulate physiological conditions.[28].

3.2. Dissolution of the neutral drugs, danazol and fenofibrate

The solubility of drugs classified under the BCS class IIc or neutral drugs is not dictated by the pH in their environment. The oral absorption is therefore largely influenced by their dissolution rate and the amount of time they spend in the GI tract.[8] Danazol is a synthetic analog of ethisterone and has been used in the treatment of endometriosis, fibrocystic breast disease, breast cancer, and hemophilia.[29] It's cholesterol-like structure makes it very lipophilic. Fenofibrate is a fibric acid derivative used to reduce elevated low-density lipoprotein cholesterol (LDL-C), total cholesterol, triglycerides, and apolipoprotein B and also increases high-density lipoprotein cholesterol (HDL-C).[30] Both are neutral compounds, and have low aqueous solubility, but have high permeability.

The dissolution of danazol in both the conventional buffer and the biorelevant buffer is shown in Fig. 4. Generally, in both instances, the amount of danazol dissolved in the stomach falls within the same range. In SGF, the concentration of danazol ranges from 1.1 – 3.8 μg/mL or an overall 0.7 % dissolved while in FaSSGF, the range is 0.7 – 2.6 μg/mL or

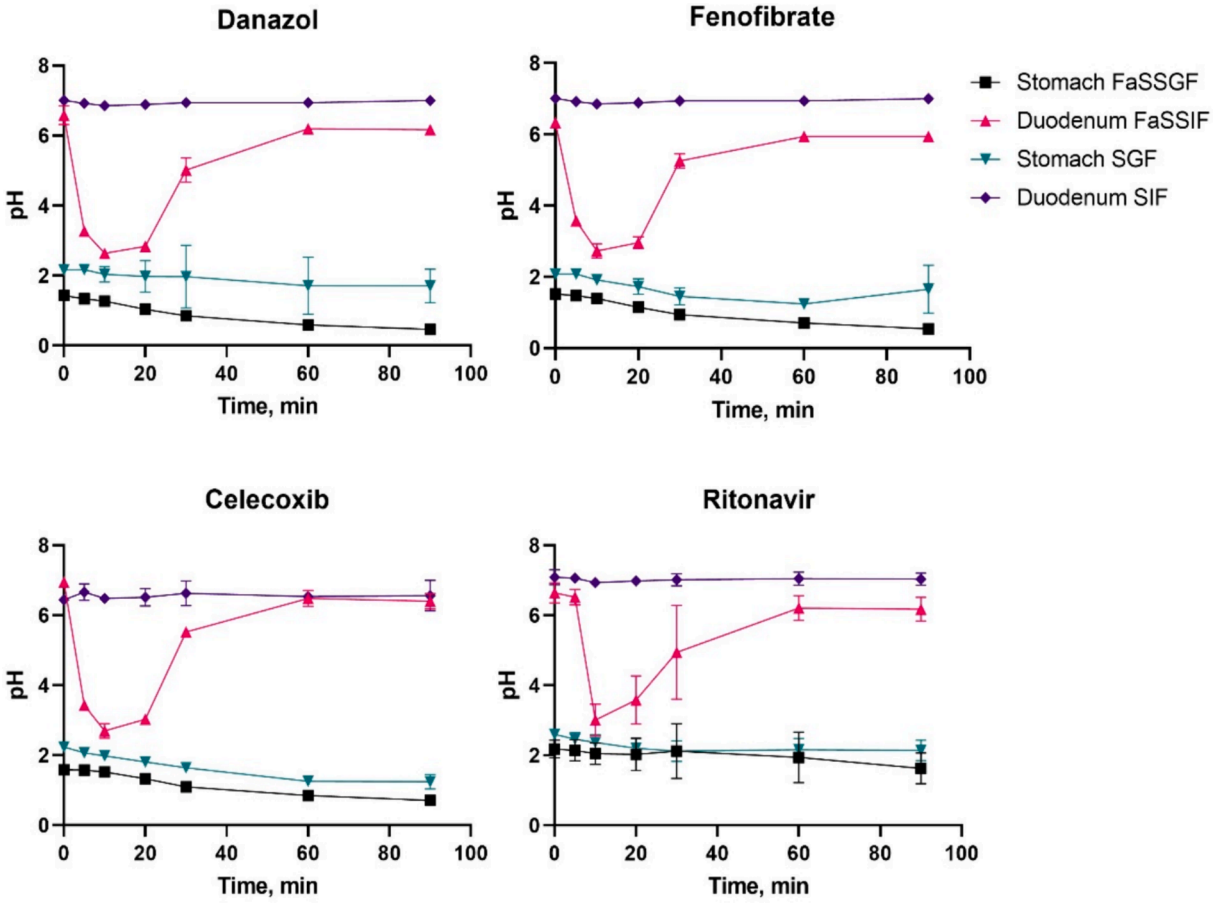

Fig. 3. Ph measurements in the stomach and duodenum for all medium used. reported data is the mean of three trials ± the standard error. In some cases, the error is smaller than the symbol used.

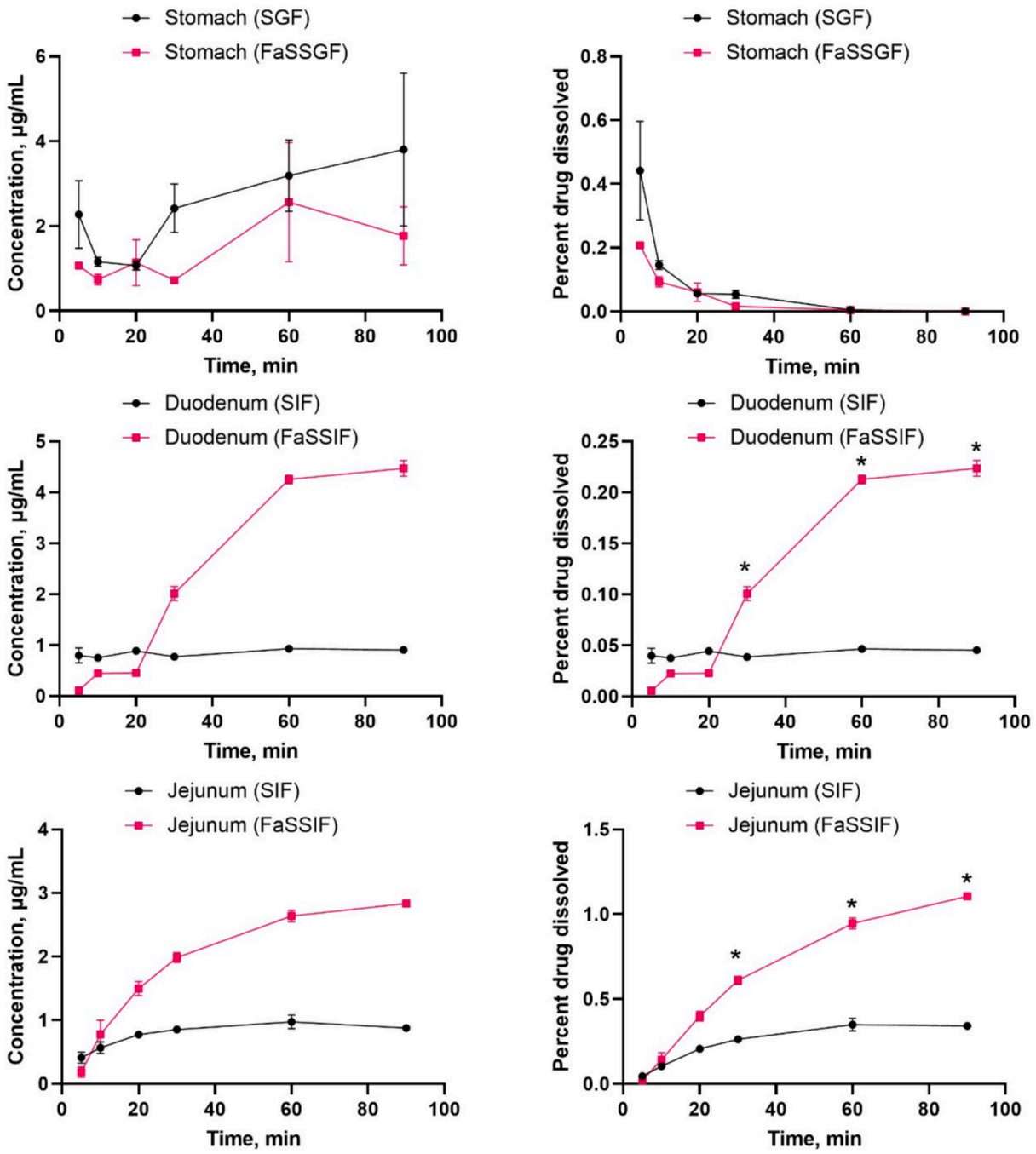

Fig. 4. Dissolution profile of Danazol (100 mg capsule) in conventional buffers, SGF and SIF, and biorelevant buffers, FaSSGF and FaSSIF, taken using the GIS-α. Data points are the mean of three trials reported with the standard error. Statistical significance between later time-points were calculated using unpaired Student's *t*-test (* is $P<0.05$).

up to 0.4 % dissolved. The difference between the conventional and the biorelevant buffer conditions is more evident in the intestinal compartments. In both the duodenal and jejunal chambers, the percentage of danazol dissolved in SIF is almost constant but in FaSSIF, the change is more drastic over time reaching up to more than 1 % dissolved in the jejunum at the 90 min mark. This is due to the components of the FaSSIF buffer that aids in the solubilization of drug particles that enter the duodenal chamber (Fig. 4).

The trends observed in the dissolution of danazol can also be observed in the case of fenofibrate shown in Fig. 5. The dissolution in the stomach for both conventional and biorelevant buffers are within the same range. The trend starts to diverge in the duodenal and jejunal chambers where the dissolution rate in the biorelevant media is greater than the simple media. In the duodenum, the highest concentration reached was 0.5 µg/mL (0.02 %) when using SIF and 6.1 µg/mL (0.2 %) when using FaSSIF signifying at least 10 × improvement in the percent dissolved in solution. The use of both FaSSGF and FaSSIF provides a more accurate picture of in vivo dissolution by modeling the buffer capacity and surfactants like bile acids that is present in vivo.[31–33] The use of either SGF or FaSSGF in the gastric compartment seems to have no effect on the initial dissolution of both compounds. Typically, the pH for the fasted stomach varies from 1.2 to 2.7 and this was achieved by both buffers.[34,35] FaSSGF at pH 1.6 contains physiologically relevant amounts of bile salts and lecithin to obtain biorelevant surface tension.

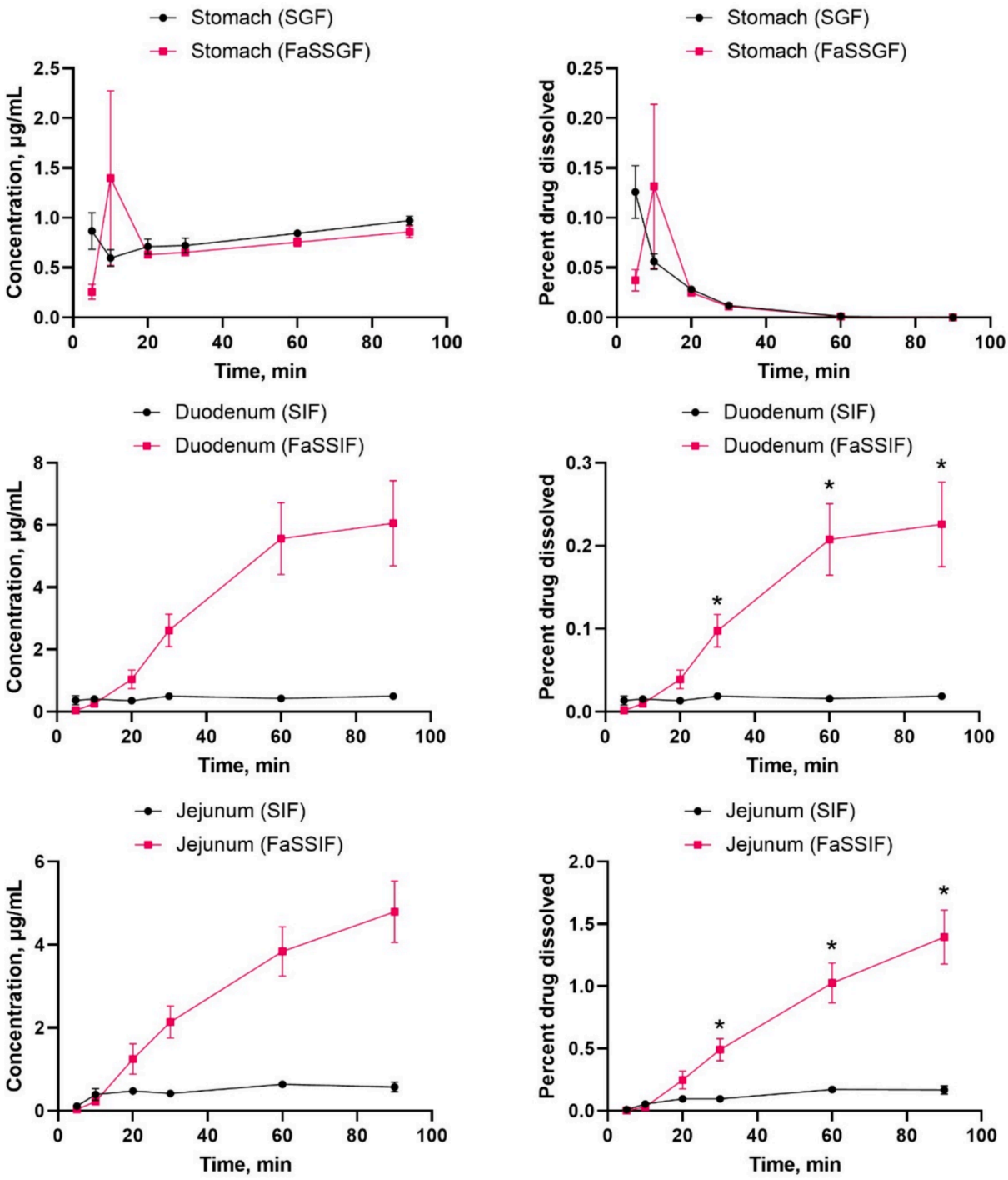

Fig. 5. Dissolution profile of Fenofibrate (134 mg capsule) in conventional buffers, SGF and SIF, and biorelevant buffers, FaSSGF and FaSSIF, taken using the GIS-α. Data points are the mean of three trials reported with the standard error. Statistical significance between later time-points were calculated using unpaired Student's *t*-test (* is $P<0.05$).

[36] SIF is designed to have a higher buffer capacity that is useful in maintaining the desired pH in the small intestinal compartment. In addition, SIF is generally used for quality control purposes and has a limited in vitro-in vivo correlation (IVIVC) capabilities.[36] On the other hand, FaSSIF has a lower buffer capacity allowing it to change pH as it is mixed with the gastric juices.[37] This is evident in the pH readings obtained from the experiment. The GI lumen has been shown to be buffered by bicarbonate, however the use of bicarbonate buffers is not pragmatic because of the thermodynamic complexity between the participating ions and its lower buffer capacity.[38] One would need to constantly replenish CO_2 to keep the buffer at the desired pH level or use a floating lid to prevent the interaction with the air and the evaporation of CO_2.[39] The SIF media achieved saturation of test compound much faster than the FaSSIF media. This agrees with reports that phosphate buffers resulted to faster disintegration times than biorelevant buffers.[40] This further solidifies the use of biorelevant buffers for modeling BCS class II drugs.

3.3. Dissolution of the weakly acidic drug, celecoxib

The benzene sulfonamide drug, celecoxib (Fig. 2), is an orally administered non-steroidal anti-inflammatory drug (NSAID) used for the treatment of patients with rheumatism and osteoarthritis. It works by selectively inhibiting cyclooxygenase-2 (COX-2) which is one of the enzymes responsible for prostaglandin synthesis and has implications in pain and inflammation.[41] It is classified under the BCS class IIa because of its poor solubility, high permeability, and an acid dissociation constant of 10.7.[10,19] Being a weak acid means that the degree of ionization will be affected by the pH of its environment and it will have higher solubility at higher pH values. One would expect a lower percentage of drug dissolved in the stomach than in the intestine. Indeed, this is what was observed in its dissolution profile shown in Fig. 6. About 0.1 % of celecoxib was detected in the stomach both for the SGF and the FaSSGF. This value went up to 1.6 % (16.5 μg/mL) for FaSSIF and 0.3 % (3.4 μg/mL) for SIF in the jejunal compartment after 90 min. This observation also correlates with the observed drop in pH in the duodenal compartment for FaSSIF where the amount dissolved increased steadily as the pH went up.

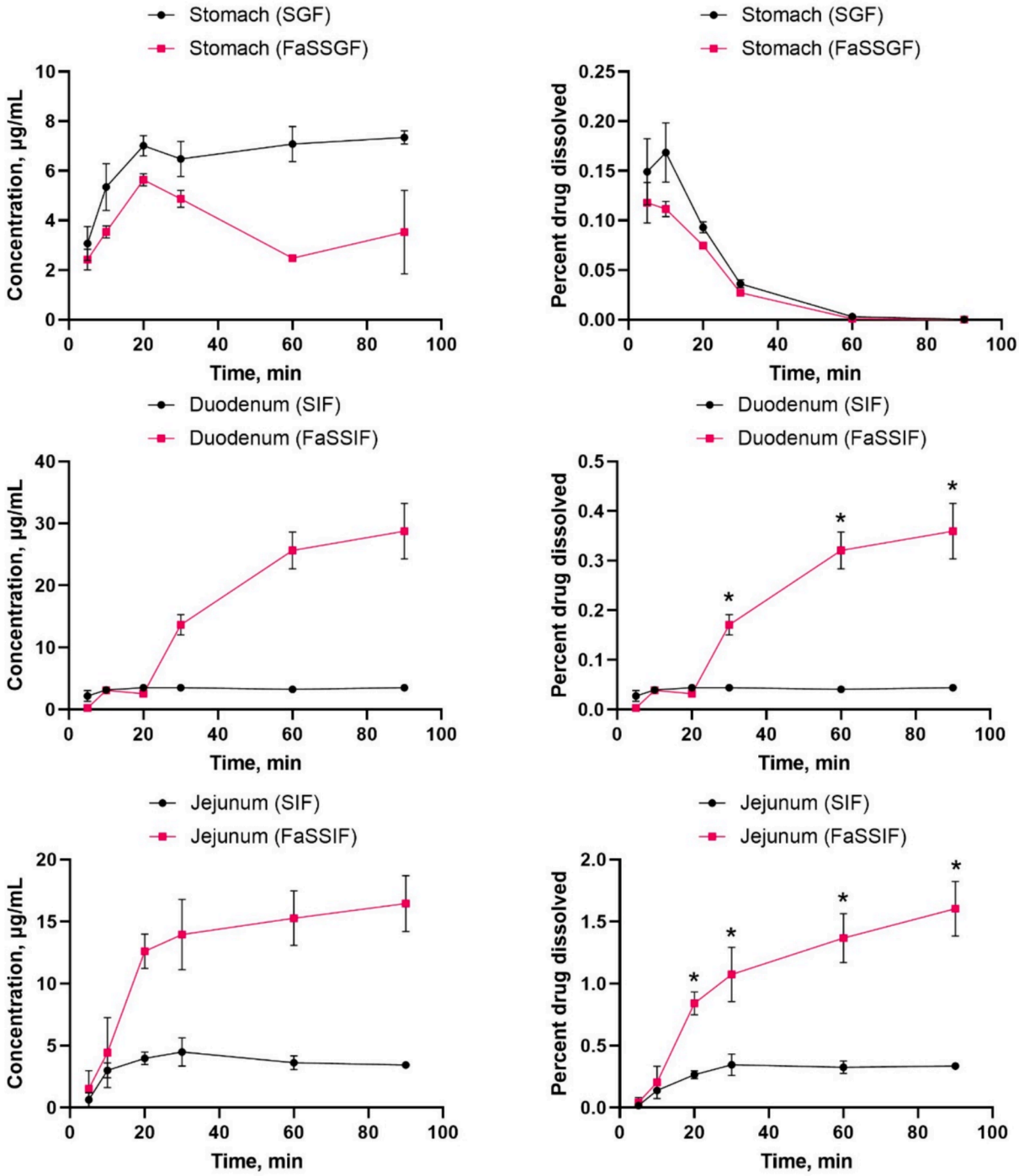

Fig. 6. Dissolution profile of Celecoxib (400 mg capsule) in conventional buffers, SGF and SIF, and biorelevant buffers, FaSSGF and FaSSIF, taken using the GIS-α. Data points are the mean of three trials reported with the standard error. Statistical significance between later time-points were calculated using unpaired Student's *t*-test (* is $P<0.05$).

Celecoxib has a very poor solubility in acidic media that the observed amount dissolved did not exceed 1 % as demonstrated in the results of this study. This solubility is expected to increase with pH, however, the high pK_a value of 10.7, which is outside of physiological pH, renders deprotonation to be at a minimum at the pH value of the small intestines. [10] Chemical prediction using ADMET Predictor yields another pK_a value for Celecoxib at −2.9 which is also outside the physiological pH range. This means that the uncharged state of Celecoxib is the main species present throughout the experiment. The absorption of celecoxib is severely limited by its solubility resulting to a variable bioavailability. [42].

3.4. Dissolution of the weakly basic drug, ritonavir

Weakly basic drugs act in the opposite way as weak acids. Their solubility is greater when in a low pH environment such as the stomach and it decreases as the pH goes up in the intestine.[8] Ritonavir is originally intended to be a human immunodeficiency virus (HIV)

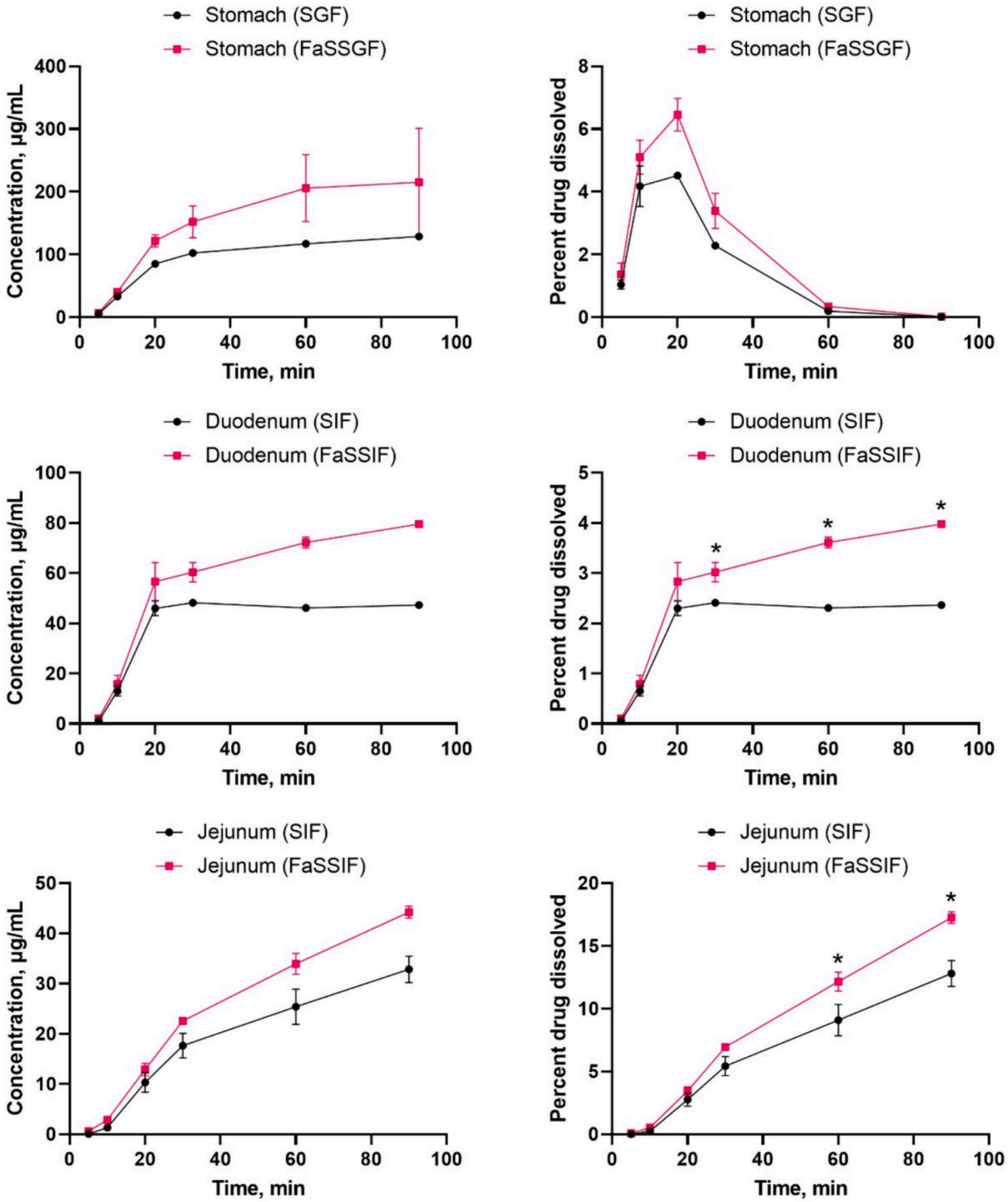

Fig. 7. Dissolution profile of Ritonavir (100 mg tablet) in conventional buffers, SGF and SIF, and biorelevant buffers, FaSSGF and FaSSIF, taken using the GIS-α. Data points are the mean of three trials reported with the standard error. Statistical significance between later time-points were calculated using unpaired Student's *t*-test (* is $P<0.05$).

protease inhibitor used for the treatment of autoimmune deficiency syndrome (AIDS).[43] In recent years, the value of ritonavir has extended to serve as P-glycoprotein and CYP3A4 inhibitor, as a combination therapy with anti-virals for treatment of hepatitis C, and is also currently undergoing studies for its use against the severe respiratory syndrome coronavirus 2 (SARS-CoV-2).[9,44] Ritonavir is also practically insoluble in water hence its absorption is potentially limited by its dissolution rate.

Fig. 7 shows the dissolution of ritonavir in GIS-α using the conventional buffers and the biorelevant buffers. As a weak base drug, it can be expected that it will start dissolving in the stomach environment than in the intestine. This is exactly what was observed in the results. There is 215.15 μg/mL ritonavir dissolved in the gastric chamber in the FaSSGF media which is higher than the 79.5 μg/mL observed in the duodenal chamber and 44.2 μg/mL in FaSSIF after 90 mins of dissolution. The same trend is observed when using simple buffers. This can be attributed to the two weakly basic thiazole moieties with pK_a values of 1.8 and 2.6.[43] With this, ritonavir would be readily ionized in the gastric environment, thus becoming soluble, but would primarily exist in un-ionized state in the higher pH of the small intestinal environment like the duodenum and jejunum. This may cause precipitation to occur causing a lower concentration to be detected. As time goes on, the amount of ritonavir dissolved in both conventional and biorelevant media in the jejunal chamber goes up with only a ~ 5 % difference between them. It is also worth noting that the dissolution of ritonavir in the simple media and the biorelevant media are almost similar in all three compartments of the GIS-α with only a small difference.

3.5. Comparison with USP II dissolution

To assess the suitability of the GIS-α in studying the dissolution of BCS class II drugs, we obtained the dissolution profiles of the model drugs using the USP II dissolution apparatus. We opted for the use of 500 mL SIF as opposed to the usual 900 mL to get closer to the 300 mL total volume used in the GIS-α gastric compartment and without moving too far from the US Pharmacopoeia standards. Results are shown in Fig. 8.

The USP apparatus is traditionally used to do quality control and batch-to-batch comparison of pharmaceutical products. They are also used to provide biowaiver eligibility especially for immediate release (IR) oral drug formulations belonging to the BCS class I.[45] However, these apparatuses are generally considered not suitable for assessment of poorly soluble drugs. This is because the solubility and dissolution of these drugs are largely dependent on the physiological factors of the GI tract such as the variable pH, motility, and many more.[13,28] The observed dissolution of fenofibrate, danazol, and celecoxib is greater in the biorelevant media than the conventional media and the USP II. This is expected since the USP II dissolution does not consider some of the complexities of the in vivo condition such as the solubilization effect by bile acid components in FaSSIF. Since dissolution is so low, the improvement caused by using FaSSIF is very evident. For fenofibrate and danazol, the conventional GIS-α dissolution performed slightly better than the USP II. This means that the dissolution of both compounds in the gastric compartment slightly affected the outcome and is better than performing the dissolution in a single compartment. This could be due to the formulation itself as the capsules could contain excipients that would help in the dispersion of drug particles in the stomach. In the case of celecoxib, the two methods are almost identical with no significant difference between them. Being a weak acid, celecoxib did not benefit from the acidic environment in the gastric compartment and only achieved most of its dissolution in the duodenal and jejunal compartments. This could explain the similar dissolution profile between the USP II and the GIS-α using SIF media. These results align well with reports saying that the use of in vivo predictive methods, like the GIS-α, and combining that with the use of biorelevant buffers could better predict dissolution of test medications.[32,33,35].

The most striking observation was found in ritonavir where the biorelevant dissolution is only slightly better than the conventional GIS-

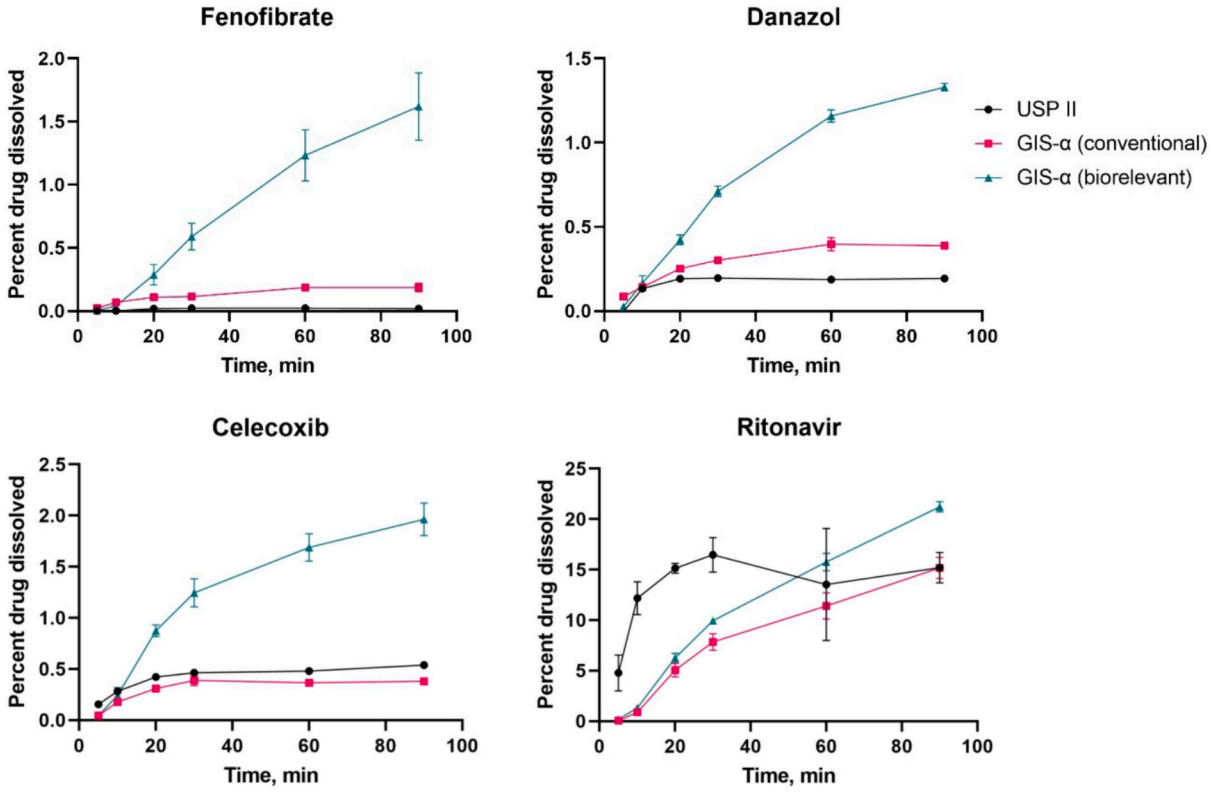

Fig. 8. Comparison of dissolution methods used in this study.

α. The USP II also performed better at the initial time points (up to 30 mins) which can be attributed to the larger volume of media used compared to the conditions in the GIS-α. Being a weak base, the dissolution will be more favorable in the acidic environment of the stomach and would suffer from supersaturation and subsequent precipitation once it reaches a higher pH environment.[4,7] This can be observed in the GIS-α but not with the USP II. The USP II methodology only utilizes a single compartment and while the observed amount of drug dissolved exceeded the percentage observed in the GIS-α at earlier timepoints, there is an evident decrease in amount dissolved after 30 mins suggesting that it reached supersaturation that also led to precipitation.

3.6. Simulations using GastroPlus™

Convolution of drug dissolution data to obtain or predict the pharmacokinetic profile of a drug aids in designing oral formulations to get the desired outcome.[46] To assess how well the dissolution profiles obtained from both the GIS-α and USP II fare with clinical data, GastroPlus™ was utilized. This modeling approach is done not to accurately predict the plasma profiles but to get the rank order of dissolution profiles obtained by conventional dissolution methodologies and transfer dissolution methodologies with different buffer species. Fig. 9 summarizes the results. The physicochemical properties listed in Table 1 was used as input alongside the in vitro dissolution data and the resulting simulation was taken without further optimization for comparison purposes.

The calculated and literature-derived pharmacokinetic parameters are listed in Table 2. From Fig. 9, even with the use of biorelevant buffers, the GIS-α, in its current form, underpredicted in vivo plasma concentration for danazol, fenofibrate, and celecoxib. However, the overall prediction is still better than when using conventional buffers or the USP II. The prediction for ritonavir even came close to clinical data available in the literature. This is expected given the faster dissolution profile obtained for ritonavir in the GIS-α. The USP II dissolution of ritonavir also gave a higher dissolution rate but eventually reached supersaturation after 30 mins. This led to the lower value of C_{max} (0.21 μg/mL) and T_{max} (2 h) obtained compared with the results from the GIS-α. There are a few potential reasons that the plasma profiles of those model drugs all underpredicted: 1) the low solubility/dissolution data as inputs. It is hard to determine drug solubility for especially, lowly soluble drugs in the physiological pH range (Table 1). 2) The variability in the clinical data as shown in Table 2. and 3) Physiological condition and solubilization effects in the in silico software. As the solubilization effect of bile acids was demonstrated in the dissolution studies between FaSSIF and SIF, as well as the pH dependent solubility. This dynamic phenomenon of bile acid solubilization is hard to capture and incorporate into the prediction because the secretion rate of bile acid, and its concentration would be different in patients as well as pH and aqueous volume in the gastrointestinal tract. Therefore, this is one of limitations in the software to predict the drug absorption for lowly soluble drugs. In general, the more physiological conditions included in the model/system, the better the predictions will be. The USP II method represents a static model with a controlled pH parameter offered by SIF. The GIS-α models the material transfer from one GI compartment to the other while also keeping physiologically relevant liquid volume in the gastric and duodenal compartments. When using biorelevant media, another layer of complexity added in that fasting state buffer conditions is met by using FaSSGF and FaSSIF.[28] This is showcased in the results where it is seen that the biorelevant GIS-α performed better than the other dissolution condition and method.

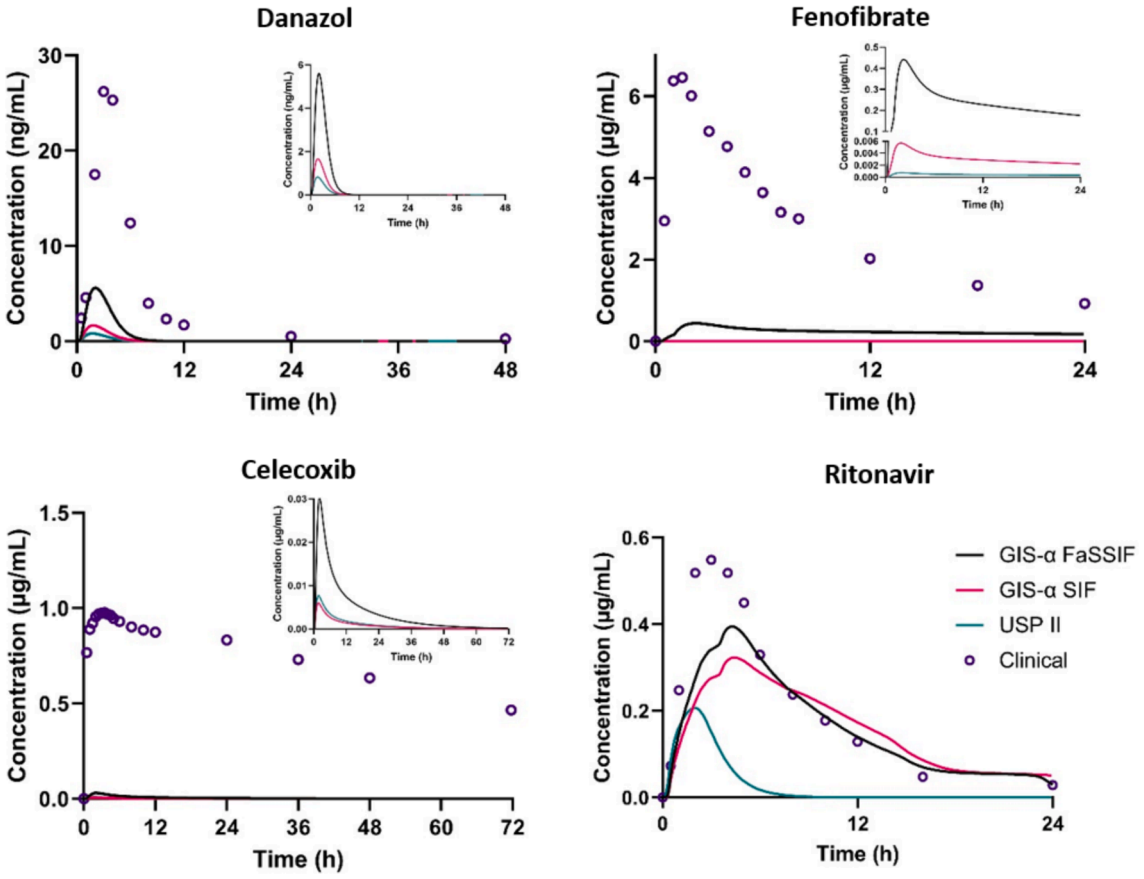

Fig. 9. Predicted plasma concentration–time profile for all drugs tested. Clinical data for danazol (100 mg), fenofibrate (145 mg), celecoxib (400 mg), and ritonavir (100 mg) are represented as open circles and included for visual comparison.

Table 2
Pharmacokinetic parameters obtained from GastroPlus™ simulations. (Clinical data was shown as mean ± s.d.).

		C_{max} (μg/mL)	T_{max} (h)	$AUC_{0-\infty}$ (μg-h/mL)	Fa (%)
Danazol	FaSSIF	5.60	2.08	18.58	1.4
	SIF	1.66	1.86	5.37	0.4
	USP II	0.82	1.70	2.63	0.2
	Clinical (n = 8)[a]	25 ± 17	3.1 ± 0.7	120.0 ± 60.0	11.0 ± 5.2
Fenofibrate	FaSSIF	0.44	2.24	13.94	14.56
	SIF	5.7×10^{-3}	1.92	0.18	0.18
	USP II	7.5×10^{-4}	1.92	0.03	0.03
	Clinical (n = 8)[b]	7.5 ± 1.5	1.4 ± 1.1	91.1 ± 34.0	68.8 ± 10.8
Celecoxib	FaSSIF	0.03	2.10	0.29	2.0
	SIF	5.99×10^{-3}	1.78	0.06	0.4
	USP II	7.72×10^{-3}	1.78	0.07	0.5
	Clinical (n = 50)[c]	1.8 ± 3.5	4.5 ± 1.0	75.4 ± 4.3	100*
Ritonavir	FaSSIF	0.39	4.24	3.90	87.7
	SIF	0.32	4.40	4.20	86.4
	USP II	0.21	2.00	0.68	15.2
	Clinical (n = 27)[d]	0.6 ± 0.3	3.2 ± 1.2	4.6 ± 2.0	>60-80[e]

* Mean Relative Bioavailability[42].
[a] [29].
[b] [47].
[c] [48].
[d] [21].
[e] [49].

4. Conclusions

In the absence of in vivo tests, a very good dissolution method is the only analytical method that can be used to evaluate performance of oral formulations.[50] The use of GIS-α with biorelevant buffers is a suitable way of generating dissolution profiles that can be used for predicting in vivo performance of drugs belonging to BCS class II. It must be noted, however, that the results with FaSSGF are not far from the results obtained with SGF in the gastric chamber. On the other hand, there is a significant difference between FaSSIF and SIF. This can be due largely to the buffer capacity of FaSSIF that allows it to mimic the in vivo change in pH experienced by the intestines as it receives gastric juices from the stomach as well as the solubilization effect by the bile acid components that are present. The USP II dissolution performed poorly in all cases. The GIS-α also provides more dynamic information because it can model dissolution events in different parts of the GI tract that can be otherwise missed when using a single compartment dissolution model such as the USP II. For example, the greater dissolution of ritonavir in the stomach may have been missed. The general trend observed in this study is: biorelevant GIS-α > conventional GIS-α > USP II, reflecting that the closer we get to physiological conditions, the closer we get to actual clinical outcome. The results, however, are still far from perfect. Nonetheless, the result for ritonavir is promising and this has the potential to be applicable to BCS class IIb drugs.

The GI tract is complex and one of the factors missing in the GIS-α setup is the lack of an absorptive sink, especially in the intestinal compartments. In the current setup, supersaturation can be reached, and precipitation can happen which will lead to a lower percentage of drug dissolved. Incorporation of a biphasic setup may be beneficial as demonstrated in an in vitro dissolution study involving donepezil.[7] Motility and mixing inside the GI compartments must also be carefully taken into account as it also plays a role in how the contents are broken down. However, this is highly variable between individuals and between feeding states and is therefore harder to incorporate into a simplified model. Optimization of this in vitro predictive methodology is needed in order to achieve a clinically relevant dissolution results that can be applied to low solubility oral drugs.

CRediT authorship contribution statement

Marvin D. Naing: Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Yasuhiro Tsume:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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