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In vitro and *in silico* Dissolution and Permeation Assessment

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To my family.

“A smooth sea never made a skilled sailor”

Franklin D. Roosevelt

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Abstract

New chemical entities (NCEs) under development tend to be progressively more poorly water soluble. As conventional dissolution tests are not representative of *in vivo* conditions and thus not predictive of its *in vivo* behavior, formulation of these orally administered drug products is often compromised. The design of a biorelevant dissolution method reflects the physiological conditions in the gastrointestinal (GI) tract, possessing a biological discriminative power given e.g. by the increased solubilization of drug molecules by bile salts and lecithin, which is of significant importance when evaluating the dissolution behavior of poorly soluble drugs. Moreover, there has been an increasing trend in the pharmaceutical industry to use mechanistic models to complement *in vitro* data that are an inexpensive and fast way of assisting the formulation process.

The present work aims at using a biorelevant dissolution methodology to support drug product development, employing USP Apparatus 2 and different formulations (enteric and non-enteric polymers, different binders and granule sizes) of tablets of spray dried dispersions (SDDs) of Itraconazole (ITZ), a poorly water-soluble drug. SDDs, tablets and the reference commercial product dissolution were assessed in biorelevant media and a biorelevant pH shift was performed. Also, an attempt was made to simultaneously evaluate dissolution and *in vitro* permeation of ITZ, using the reverse dialysis membrane methodology. Finally, an *in silico* model describing dissolution phenomena of amorphous active pharmaceutical compounds (APIs) was developed.

Crystalline ITZ solubility in biorelevant media could not be assessed, since it was below the limit of quantification of the employed method. SDDs could not be properly tested in USP Apparatus 2 due to the characteristic poor wettability of these powders, that led to powder floating. The potential for higher bioavailability of solid oral ITZ through intestinal targeting was demonstrated via pH shift. It was not possible to quantify the molecularly dissolved ITZ through reverse dialysis method, which lacks further development and optimization. The obtained results show that the compendial dissolution methodology is not enough to evaluate poorly-soluble dosage forms performance because they can often lead to a sub or over estimation of its solubility.

Keywords: *in vitro, in silico*, dissolution, biorelevant, poorly-soluble-drugs, free-drug

Resumo

Os novos princípios ativos em desenvolvimento tendem a ser cada vez mais hidrofóbicos. Uma vez que os testes de dissolução convencionais não são representativos das condições *in vivo*, não permitindo prever o seu comportamento nestas mesmas condições, a etapa de formulação destes fármacos poderá ser inglória. A conceção de um método de dissolução biorelevante reflete as condições fisiológicas do trato gastrointestinal, possuindo uma capacidade de discriminação biológica, por exemplo devido à maior solubilização do fármaco por sais biliares e lecitina, relevante para o estudo da dissolução de fármacos pouco solúveis em água. Para além disto, tem havido uma tendência na indústria farmacêutica para utilizar modelos mecanísticos para complementar os dados *in vitro*, que constituem uma estratégia rápida e fácil de auxiliar o processo de formulação.

Este trabalho visa utilizar a metodologia de dissolução biorelevante para suportar o processo de formulação de fármacos com pouca solubilidade aquosa, recorrendo ao equipamento de dissolução *USP Apparatus 2* e diferentes formulações (polímeros entéricos e não entéricos, ligantes e tamanhos de grânulos diferentes) de comprimidos com dispersões sólidas amorfas (DSAs) de Itraconazole (ITZ), um fármaco pouco solúvel em água. A dissolução das DSAs, dos comprimidos e do produto comercial (referência) foram estudados em meios biorelevantes e foi realizado um ensaio de dissolução biorelevante com transição de pH para as formulações mais promissoras. Também foi feita uma tentativa de quantificar simultaneamente a dissolução e permeação *in vitro* do ITZ usando o método de diálise reversa. Por último, desenvolveu-se um modelo *in silico* para descrever o fenómeno de dissolução de princípios ativos (PAs) amorfos.

Não foi possível determinar a solubilidade do ITZ cristalino, por estar abaixo do limite de quantificação do método utilizado. A dissolução das DSAs no *Apparatus 2* não foi avaliada nas condições mais adequadas, devido à baixa molhabilidade típica destes produtos que, por conseguinte, flutuam no meio de dissolução. Demonstrou-se o potencial de maior biodisponibilidade de uma formulação entérica de ITZ via dissolução com mudança de pH. Não foi possível quantificar o ITZ livre em solução pelo método de diálise reversa, sendo que este carece de futuro desenvolvimento e otimização. Os resultados obtidos mostram que os métodos de dissolução compendiais não são suficientes para estimar o desempenho de fármacos pouco solúveis *in vivo*, uma vez que estes levam frequentemente a uma sub ou sobrestimação da sua solubilidade.

Palavras-chave: *in vitro*, *in silico*, dissolução, biorelevante, fármacos-pouco-solúveis, fármaco-livre.

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List of Acronyms

(m)DSC	(modulated) Differential Scanning Calorimetry
API	Active Pharmaceutical Ingredient
ASD	Amorphous Solid Dispersion
BCS	Biopharmaceutical Classification System
CE	Cellulose Ester
DCM	DiCloroMethane
DCS	Developability Classification System
EP	European Pharmacopeia
FaSSGF	Fasted State Simulated Gastric Fluid
FaSSIF	Fasted State Simulated Intestinal Fluid
FDA	Food and Drug Administration
FeSSGF	Fed State Simulated Gastric Fluid
FeSSIF	Fed State Simulated Intestinal Fluid
FIP	International Pharmaceutical Federation
GI	Gastrointestinal
HIV	Human Intestinal Fluid
HPLC	High Performance Liquid Chromatography
HPMC	HydroxyPropyl MethylCellulose
HPMCAS LF	HydroxyPropyl MethylCellulose Acetate Succinate L and Fine powder grade
IDR	Intrinsic Dissolution Rate
IR	Immediate Release
ITZ	Itraconazole
IVIVC	*In Vitro In Vivo* Correlation

JP	Japanese Pharmacopoeia
LOQ	Limit Of Quantification
MCC	Microcrystalline cellulose
MRT	Mean Residence Time
MWCO	Molecular Weight CutOff
NCE	New Chemical Entity
PSD	Particle Size Distribution
PVP/VA	PolyVinylPyrrolidone-Vinyl Acetate copolymer
QC	Quality Control
RCS	Refrigerated Cooling System
RSF	Relative Span Factor
SD	Spray Drying
SDD	Spray Dried Dispersion
SDI	Spray Drying Intermediate
SDS	Sodium Dodecyl Sulphate
SEM	Scanning Electron Microscopy
SGFsp	Simulated Gastric Fluid sine pepsin
SIFsp	Simulated Intestinal Fluid sine pepsin
SPO	Sporanox®
TPGS	d-α-Tocopheryl Polyethylene Glycol 1000 Succinate
UPLC	Ultra Performance Liquid Chromatography
USP	United States Pharmacopeia
XRPD	X-Ray Powder Diffraction

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Introduction

1.1. Motivation

Oral drug delivery represents the primary route for drug administration [1], due to its convenience of self-administration, ease of handling of the dosage form by the patient and lower cost of the final drug product in contrast to, e.g., sterile manufacturing for injectables [2]. However, in order to achieve therapeutically effective concentrations upon oral administration, the drug must exhibit satisfactory biopharmaceutical properties.

In a drug discovery setting, oral bioavailability, which is defined as the fraction of an oral dose of the drug that reaches the systemic circulation, is the pharmacokinetic measure of drug candidate suitability for oral administration most commonly used [2]. Drug absorption at the intestine is the result of a number of steps (Figure 1.1), including drug dissolution in the GI tract and uptake through the intestinal mucosa, followed by delivery into the systemic circulation. In order to predict the *in vivo* performance of a drug after oral administration, it is critical to establish the physiochemical and physiological factors affecting drug absorption [3]. An high oral bioavailability reduces the amount of administered drug necessary to achieve a desired pharmacological effect, thus avoiding potential side-effects and toxicity associated to higher doses and can reduce inter-individual variability, averting an unpredictable response to a drug [2].

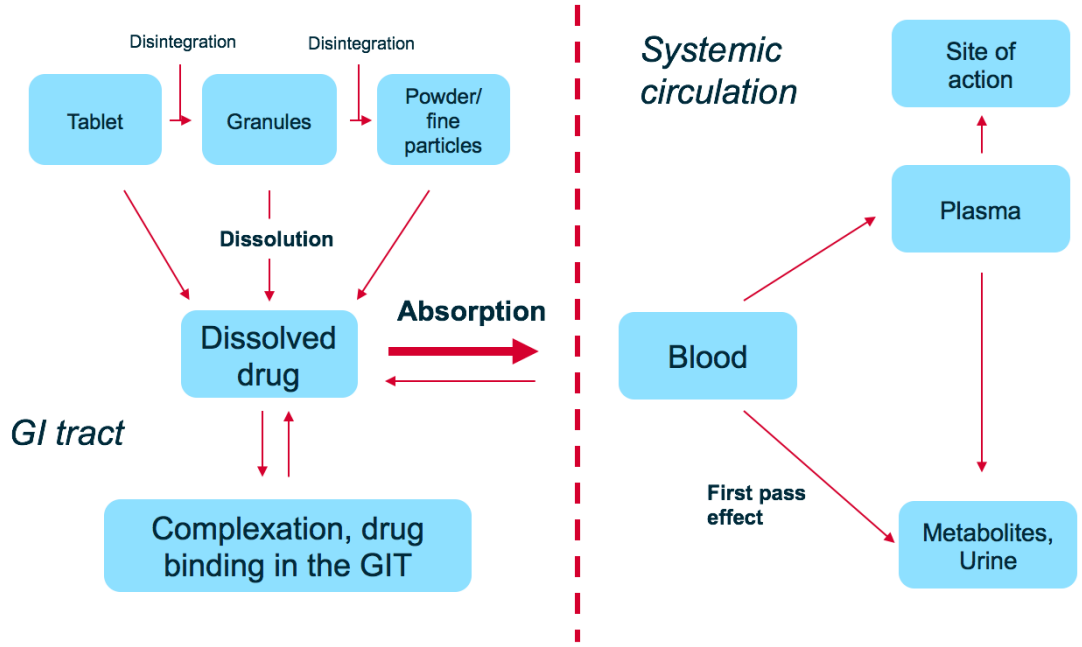


Figure 1.1 – Dissolution and Oral Drug Delivery. Adapted from [4]**.**

Dissolution testing is an important analytical tool that is widely used by the pharmaceutical industry to assess and establish product behavior during the various stages of drug product development, as well as life cycle management. It is described in is described in many pharmacopeias, e.g. European Pharmacopeia (EP) and United States Pharmacopeia (USP) and Food and Drug Administration (FDA) guidelines.

The relationship between *in vitro* dissolution and *in vivo* performance is based on the fact that the active pharmaceutical ingredient (API) must be in solution in the GI tract so that it can be available for absorption. For a proper use in the evaluation of dosage forms performance, the quality of the *in vitro* dissolution data is of paramount importance. Thus, the main goal of a dissolution test should be a discriminatory method, sensitive to variables that impact the release rate and are simultaneously representative of the *in vivo* performance (Figure 1.2). Such variables may include characteristics of the API, drug product formulation and drug product manufacturing and effects of stability storage conditions [3]–[5].

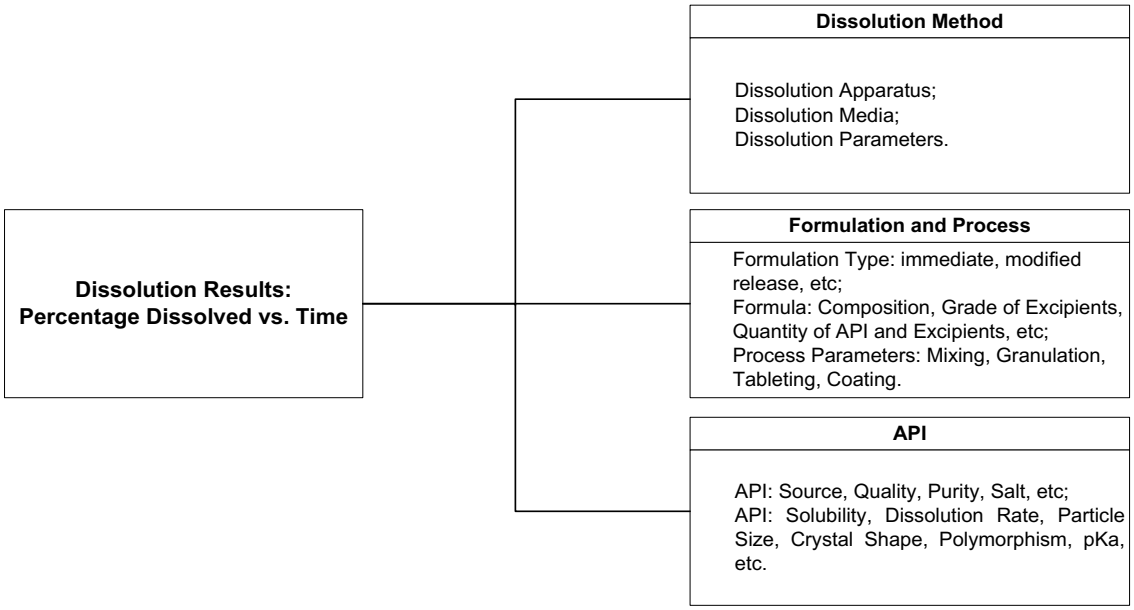


Figure 1.2 – Factors influencing *in vitro* dissolution [5]**.**

At the early stages of development, also referred as preformulation, dissolution testing of pure APIs serves as a tool in the screening of physicochemical properties of drug candidates [6]. It guides the selection of toxicology and phase I formulations for evaluation in animals and humans. At later stages of development, the dissolution tests are performed with drug products to compare prototype formulations, elucidate the drug release mechanisms, assess the stability of the formulated product and the robustness of the manufacturing process to ensure safety and reproducibility of the products released to the market [7].

During the development of a pharmaceutical product, dissolution testing is used as a tool to identify formulation factors that influence and may have a significant effect on the bioavailability of the API. After formulation and manufacturing processes are defined, dissolution testing is used in the quality control of scale-up and of production batches, ensuring batch-to-batch consistency. A quality control (QC) release dissolution test is overtly discriminatory with no relevance to the *in vivo* performance of the product. As conventional dissolution tests in USP Apparatus 1 and 2 described in the pharmacopoeias use a constant fluid volume, pH and buffer species, which are not physiologically relevant of the human GI tract, formulation development of poorly soluble drugs is often hindered. Accordingly, the prediction of the *in vivo* performance of these oral drug products is compromised.

The rate and extent of absorption of an oral dosage form are primarily controlled by two factors: solubility and permeability. In this regard, FDA established the Biopharmaceutics Classification System (BCS). According to BCS, drug substances are divided in different classes I, II, III or IV according to their solubility and permeability (Figure 1.3). This system can be used to flag a drug that should not be tested clinically unless appropriate formulation strategies are employed.

For example, BCS Class II compounds are not likely good clinical candidates without the use of enhanced formulation techniques in order to increase solubility or dissolution rate.

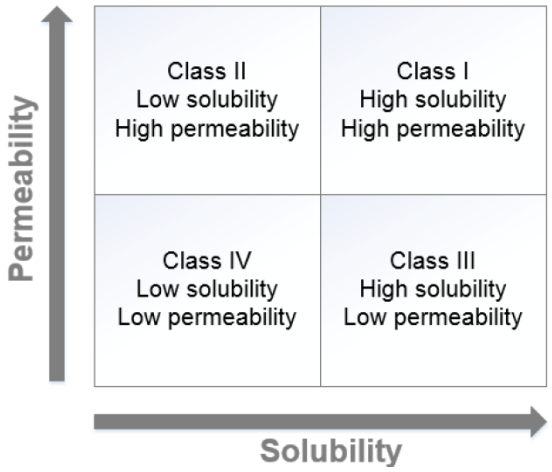


Figure 1.3 – Biopharmaceutics Classification System. Adapted from [8].

Recently, a new classification system that aims to support formulation process has been proposed – Developability Classification System (DCS) [9]. This system, although having the same classes of BCS, divides Class II in two sub-classes, IIa, dissolution rate limited drugs and IIb, solubility limited drugs, which facilitates the choice of formulation strategy, e.g. for class IIa drugs, one formulation option would be particle size reduction while for a class IIb drug, an amorphous solid dispersion would be suitable.

Most of NCEs (drugs that contain any functional group not yet known or approved by the FDA) under development are intended to be used as solid dosages forms, even though up to 90% of these entities are poorly water soluble drugs, i.e. not well-absorbed after oral administration [10]. For orally administered drugs, low aqueous solubility represents an obstacle to efficient absorption across GI tract and thus to an high oral bioavailability [1]. Therefore, one of the major current challenges of the pharmaceutical industry is to develop strategies in order to enhance water solubility and the dissolution rate of drugs [11]. Consequently, dissolution tests are particularly critical in BCS class II drugs, whose bioavailability is limited by their solubility or dissolution rate.

There are several platforms to improve the release of BCS class II drugs, which include changing the delivery strategy, particle engineering of the drug substance and/or intermediates, amorphous dispersions, complexation (e.g. with cyclodextrins), lipidic formulations and excipient optimization. Thus, it is possible to use the *in vitro* dissolution tests to identify the aspects that need to be adjusted or modified, in order to obtain the desired dissolution profile and consequently the optimal pharmacokinetic properties.

Biorelevant dissolution tests consist in dissolution tests that simulate the conditions of the GI tract and can predict the *in vivo* performance of drug products. They are useful from the early

stages of drug discovery and development through the later stages of development for identifying the biopharmaceutical performance of the drug product, solubility issues, food effects and precipitation in the small intestine. In fact, they can assist in formulation strategies and in the establishment of *in vitro in vivo* correlations (IVIVCs) which allow the reduction of the number of *in vivo* studies [12]. Particularly, biorelevant dissolution tests have a biological discriminative power which is of utter importance to poorly soluble drugs, i.e. class BCS II - where the dissolution is the rate limiting step to absorption, whereas the compendial dissolution tests are not representative of *in vivo* conditions and thus not predictive of its *in vivo* behavior. Biorelevant dissolution tests have been shown to be a useful tool to predict the absorption of poorly soluble drugs [12]–[14]. For example, food and bile components can promote the solubility of the drug, resulting in a much greater solubility *in vivo* than *in vitro* tests, using buffers as dissolution media [15].

As there is also a demand to reduce development times and increase cost-effectiveness, there is a clear need of *in silico* mechanistic models with the capability to accurate predict solubility/dissolution limited absorption. Reliable predictions on these aspects would be most useful to guide formulation development (e.g. Design of Experiments) and taking into account inter-patient variability). Additionally, computational modeling provides an inexpensive and fast way to assess the dissolution and intestinal permeability of a molecule before synthesis and also enables prioritization of molecules for *in vitro* and *in vivo* studies.

This MSc work seeks to develop dissolution methodologies suitable for BCS class II drugs and to put together an *in silico* model that will be used to best guide and select the formulation stages.

1.2. Dissolution process

Dissolution is defined as a dynamic process by which a material is transferred from a solid state to a solution per unit of time. Dissolution of a solid dosage form is composed of at least two consecutive steps: tablet disintegration followed by dissolution of the drug in the liquid media. This process is directly related with the cohesive properties of the formulated drug and the intrinsic physicochemical properties of the drug molecule [7].

Factor affecting kinetics of drug dissolution can be understood by Noyes-Whitney equation (Equation 1) proposed in 1897 [16] based on the Nernst-Brunner modifications [16], [17] . It describes the dissolution profile of a drug particle.

$$\frac{dC}{dt} = \frac{DA}{V\delta}(C_S - C) \quad \textbf{(1)}$$

where C is drug concentration, D is drug diffusion coefficient, A is drug surface area available for dissolution, δ is the thickness of the hydrodynamic boundary layer, C_s is the equilibrium solubility of the API at the solid liquid interface and V is the volume of the dissolution medium. Each parameter of the equation is influenced by physicochemical and physiological factors as described

in Table 1.1.

Table 1.1 – Physicochemical and physiological parameters influencing drug dissolution in the GIT. Modified from [18]**.**

Factor	Physicochemical parameter	Physiological parameter
Drug surface area (A)	Particle size, wettability	Surfactants in gastric juice and bile
Drug diffusivity (D)	Molecular size	Viscosity of luminal contents, diffusivity of mixed micelles
Boundary layer thickness (δ)	-	Mobility pattern, flow rate
Solubility (C_s)	Hydrophobicity, crystal structure, pKa	pH, buffer capacity, bile, food
Bulk drug concentration (C)	Particle size, wettability, solubility	Permeability
Volume of solvent available (V)		Secretions, co-administered fluid

1.3. Biorelevant dissolution methodology

The design of a biorelevant dissolution method should take into account for the following factors to reflect the physiological conditions in the GI tract: pH conditions, volume of the GI contents, characteristics of the composition of the GI contents: buffer capacity, osmolality, surface tension, viscosity and prandial state – composition of the GI content; temperature; hydrodynamics; transit times [19].

The duration of the test should reflect the residence times in the GI tract and thus be chosen according to the type of release of the dosage form (Table 1.2). An immediate release (IR) is designed to disintegrate in the stomach whilst being transported to the small intestine, where its dissolution should be completed, so that its absorption can take place. On the other hand, delayed release dosage forms are formulated to remain intact until they reach the GI segment in which they are supposed to release the API. There is still a third category, extended release dosage forms, which are intended to ensure prolonged drug release over a large part of the GI tract. This last two are named modified-release tablets, i.e. tablets which are designed to modify the rate, the place or the time at which the API is released [20].

Table 1.2 – Typical values of MRT (Mean Residence Times) in various segments of the GI Tract of Young Healthy Volunteers [21]**.**

Segment	MRT pellets	MRT tablets (non-disintegrating)
A. Pre-prandial		
Stomach	30 min	60 min
Duodenum	<10 min	<10 min
Upper jejunum	60 min	30 min
Lower jejunum	60 min	30 min
Upper ileum	60 min	60 min
Lower ileum	60 min	120 min
Proximal colon	4-12 h	4-12 h
B. Post-Prandial		
Stomach	2-4 h	2-10 h
Duodenum	<10 min	<10 min
Upper jejunum	60 min	60 min
Lower jejunum	60 min	60 min
Upper ileum	60 min	60 min
Lower ileum	60 min	60 min
Proximal colon	4-12 h	4-12 h

The temperature defined during a dissolution test is maintained at its physiological value, i.e. rounding 37 ºC and variations in the hydrodynamic conditions and media volumes can be obtained through the employment of different USP dissolution apparatus or within one apparatus by changing the stirring rate, reciprocating frequency, or flow rate. As for reflecting the effect of the remaining factors on the drug release, they are achieved by the use of biorelevant media.

1.3.1. Biorelevant dissolution media

Compendial media are simple aqueous preparations used to simulate mainly the pH and ionic strength of the fasted human GI compartments [22]. This type of media is essentially used for QC purposes due to its cheapness and simplicity. According to the pharmacopoeias, the pH of a dissolution medium for an oral dosage form should cover the physiological range of 1.2 to 6.8. The pharmacopeias also recommend the use of media more similar to the *in vivo* fluids – Simulated Gastric Fluid sine pepsin (SGFsp) and Simulated Intestinal Fluid sine pepsin (SIFsp) in terms of pH and ionic strength [23]. However, the compendial media do not include surfactants and therefore are less likely to simulate the solubilization and the wetting effect that occur *in vivo* due to compounds such as bile acids, bile salts, lecithin, among others, before the drug molecules are absorbed.

1.3.1.1. Media simulating the contents of the stomach

- **FaSSGF (Fasted State Simulated Gastric Fluid)**

Several attempts have been made to improve media simulating the fasting conditions in the stomach. In most of these media, particular attention was given to the simulation of the surface

tension measured in human gastric aspirates [18], [24], [25]. In these media, synthetic surfactants, such as sodium lauryl sulfate or Triton-X 100 have been utilized, although they often overestimate gastric dissolution because they induce greater solubilization effects compared to the ones occurring *in vivo* [26].

In 2005, Vertzoni et al. [27] developed Fasted State Simulated Gastric Fluid (FaSSGF) (Table 2.3) which is a dissolution medium that mimics the actual gastric composition in the fasted state according to published physiological data. This medium has a pH of 1.6 and contains physiological relevant amounts of pepsin, bile salts and lecithin in order to obtain a surface tension close to that found *in vivo*. It appears to be more biorelevant than the previously proposed media because it comprises only components that have been recovered from the fasting stomach. FaSSIF is currently recommended for biorelevant *in vitro* experiments [22].

It is not straightforward to define a standard gastric pH value, since the inter-patient variability is high. The median pH of the stomach in the fasted state falls in the range 1.5–1.9 [28]–[30], even though pH values as low as below pH 1 or as high as pH 5–6 are presented by a few patients [30].

- **FeSSGF (Fed State Simulated Gastric Fluid)**

In a fed state, the conditions in the stomach can vary widely, depending on the composition of the meal ingested. Initially, the composition of the gastric fluid will be close to the composition of the meal with respect to the pH, osmolality and the surface tension. The pH is increased to values of 3-6 [21]. Over time, with the secretion of gastric fluids and the following gastric emptying, values will return to those of the fasted state [31]. Thus, the contents of the fed stomach are complex, heterogeneous and have transient physicochemical characteristics.

Full fat milk (3.5%) and Ensure® Plus [32] were proposed to simulate the fed conditions in the stomach. While milk was first investigated as a dissolution medium about 20 years ago [32], [33] the use of Ensure® Plus has been established only a few years ago [32]. Both have a similar composition to the standard breakfast meal recommended by the Health and Human Services - Food and Drug Administration (HHS-FDA) for the food effects and bioequivalence studies [34]. However, milk and/or nutritional liquid products can only simulate the initial gastric conditions in the fed state. This is because the composition of the stomach contents in the fed state changes with time, as secretions, digestion and gastric emptying proceed [22]. There are two options to overcome this problem: to artificially gradually digest the milk with aliquots of an acidic solution into milk during the *in vitro* test or to use “snapshot” media, corresponding to a defined time-frame after meal ingestion [34]. Both techniques use homogenized milk (3.5% fat) as the initial medium. In the first approach, the initial medium is gradually digested by adding physiologically relevant amounts of a hydrochloric solution (HCl 1.83 M) containing 1.1 miligrams of protein (pepsin) per milliliter into the vessel from 0 to 90 min every 15 minutes [35]. Regarding the second approach,

Jantratid et al. [34] designed media reflecting the pH, buffer capacity and osmolality of the gastric contents during the first 75 min (early), from 75-165 min (middle) and after 165 min (late) following meal ingestion. These “snapshot” media can be used as sequential dissolution media in one test series (e.g. USP Apparatus 3 and 4). The “middle” medium FeSSGF – (Table 1.3) is suggested represent postprandial conditions for comparing formulations and/or predicting food effects compared with FaSSGF. It contains UHT-milk (3.5%) and acetate buffer mixed in equal volumes with a pH of 5 [35].

Table 1.3 – Composition of the media simulating the contents of the fasted (FaSSGF) and fed (FeSSGF) stomach [34].

Composition	FaSSGF	FeSSGF (middle)
Sodium Taurocholate (mM)	0.08	-
Lecithin (mM)	0.02	-
Pepsin (mg/ml)	0.1	-
Sodium Chloride (mM)	34.2	237.02
Hydrochloric Acid (mM)	25.1	-
Acetic Acid (mM)	-	17.12
Sodium Acetate (mM)	-	29.75
Milk/Buffer	-	1:1
pH	1.6	5
Osmolatily (mOsm/kg)	120	400
Buffer Capacity (mmol/l ΔpH^{-1})	-	25

1.3.1.2. Media simulating the contents of the intestine

- **FaSSIF (Fasted State Simulated Intestinal Fluid)**

FaSSIF was introduced in 1998 by Dressman et al. in order to simulate fasting state conditions in the small intestine according to physiological data [13], [18]. Apart from pH, osmolality and buffer capacity, FaSSIF takes into account the solubilizing capacity of the intestinal fluids preprandially. FaSSIF has a pH of 6.5 and contains sodium taurocholate and phospholipids in a ratio of 4:1 (Table 2.4). Later (2008), Jantratid et al. [34], have improved the composition of FaSSIF with minor modifications and proposed the FaSSIF-V2. The amount of lecithin is decreased from 0.75 mM in FaSSIF to 0.2 mM in FaSSIF–V2, the osmolality is lower in FaSSIF-V2 and maleate buffer is used instead of phosphate buffer (Table 2.4) [35]. However, it is worth noting that the surface tension of both FaSSIF and FaSSIF-V2 is considerably higher than the surface tension of human intestinal fluids (HIF) (54 vs. 33.6 mN/m) [36] which can lead to a greater *in vitro* solubility than that observed *in vivo*.

- **FeSSIF (Fed State Simulated Intestinal Fluid)**

Similarly to the fed state in the stomach, the composition of the fluids in the upper small intestine in the fed state will also be dependent on the type of food ingested, even though, in a lower extent than in the stomach. After ingesting a meal, there are changes in both the hydrodynamics and the intralumenal volume. The pH of the chyme (pre-digested, acidified mass of food that passes from the stomach into the small intestine) after a solid meal is lower than the intestinal fluid pH in the fasted state, while buffer capacity and osmolality show a sharp increase. As well as these factors, the increase in bile output could also be a major influence on the bioavailability of a drug. In addition, specific interactions between the drug and ingested food components may occur [22].

Together with FaSSIF mentioned above, a fed state simulated intestinal media – FeSSIF (Table 1.4) is also available [13]. FeSSIF simulates the fluids in the fed upper small intestine in terms of bile salt and phospholipids levels, pH, osmolarity and buffer capacity. The pH was set at 5 and by the addition of sodium chloride, the osmolality is adjusted to 635±10 mOsm/kg, while acetic acid is added to maintain a higher buffer capacity of 76 mmol/L/pH.

As for the gastric fluid, the composition of the intestinal fluid changes over time in the postprandial state. Therefore, similarly to the stomach media mentioned early, Dressman *et al*. developed three “snapshot” media: “early” FeSSIF, “Middle FeSSIF” and “Late” FeSSIF [34]. Though, a composite of the three intestinal “snapshot” media, FeSSIF-V2 (Table 2.4), is recommended for general assessment of dosage form performance in the fed state, since it is single phase, easy to prepare and facilitates analytic quantification [34]. These modifications were based on studies related to the characterization of the fed intestinal contents under conditions simulating bioavailability/bioequivalence [31] which indicated that the pH in the upper small intestine decreases rather slowly after meal intake [34]. Also, in the updated media, the presence of lipolysis products (glyceryl monooleate and sodium oleate) in the fed intestinal contents was also taken into account (Table 1.4) [37].

Therefore, FeSSGF and FeSSIF-V2 are suitable for the prediction of drug dissolution in the fed stomach and upper small intestine, respectively, whereas FaSSGF and FaSSIF-V2 are recommended to predictive dissolution studies in the fasted state [34].

Table 1.4 – Composition of the media simulating the contents of the fasted (FaSSIF-V1 and FaSSIF-V2) and fed (FeSSIF-V1 and FeSSIF-V2) intestine [34].

Composition	FaSSIF-V1	FaSSIF-V2	FeSSIF-V1	FeSSIF-V2
Sodium Taurocholate (mM)	3.0	3.0	15	10
Lecithin (mM)	0.75	0.2	3.75	2
Glycerol Monooleate (mM)	-	-	-	5
Sodium Oleate (mM)	-	-	-	0.8
Sodium Chloride (mM)	105.9	68.62	203.2	125.5
Sodium Hydroxide (mM)	8.7	34.8	101.0	81.65
Acetic Acid (mM)	-	-	144.1	-
Maleic Acid (mM)	-	19.12	-	55.02
Monobasic Sodium Phosphate (mM)	28.4	-	-	-
Pancreatin (mg/ml)	10 (optional)	-	40 (optional)	-
pH	6.5	6.5	5.0	5.8
Osmolality (mOsm/kg)	270	180	670	390
Buffer Capacity (mM/ΔpH)	10	10	76	25

1.3.2. Dissolution Apparatus

USP, EP and Japanese Pharmacopoeia (JP) contain individual chapters describing the types and specifications of dissolution apparatus. Different apparatuses, procedures and techniques are required for API or different dosage forms because of significant differences in formulation design and physicochemical properties of the drugs.

There are seven dissolution systems officially approved by USP that can be used to test several types of formulations. In particular, for oral dosage forms testing, phamacopoeial setups consist in Apparatus 1, 2, 3 e 4 and for the drug substance, the rotating and stationary disk.

1.3.2.1. IDR (Intrinsic Dissolution Rate)

The IDR is defined as the dissolution rate of pure substances under the condition of constant surface area, temperature, agitation, medium pH and ionic strength. The intrinsic dissolution tests are performed with a rotating disk holder, similar to the one proposed by Wood et al. [38] and a fixed disk system called stationary disk, described only in the USP [39]. The rotating-disk is the most used system [40] having a cavity for placing the drug, which is compacted to have a fixed geometry (Figure 1.4). The geometry and size of the exposed surface of the drug are known and the compressed drug is set in a location within the apparatus with lower hydrodynamic variability [40].

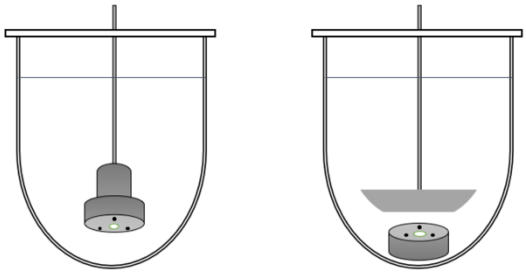


Figure 1.4 – Schematic representation of the Fixed Disk System, on the left, and of Rotating Disk System (Wood's Apparatus), on the right [40]**.**

The dissolution of pure API or active substances in preparations presented as powders or granules using USP Apparatus 4 (which will be presented in the next sections) is called apparent dissolution. In this method, the powder is simply transferred into the cell without the need for compression or compaction.

The IDR depends on the solid state properties of the API and it is useful to assess its dissolution behavior. For example, such knowledge allows to assess if the drug is solubility or dissolution rate limited.

1.3.2.2. USP Apparatus 1 and 2 - Basket and Paddle

The USP Apparatus 1 and 2 (Table 1.5) are the most commonly used dissolution equipments for drug products simulating the individual compartments of the digestive tract [6] and were proposed in the 13th edition of the USP in 1970 [41]. They are the first choice for solid dosage forms due to its easiness of operation and because they are standardized and robust.

Apparatus 1 - the basket apparatus - was described in 1968 by Pernarowski and his coworkers [42]. The assembly consists of a vessel made of glass or other inert transparent material which can be covered, a motor, a metallic drive shaft and a cylindrical basket. The vessel is typically immersed in a water bath but other heating devices can be employed. The vessel is cylindrical with a hemispherical bottom. The dosage form is placed in the basket at the beginning of each test. The basket apparatus is usually employed in dissolution testing of tablets, capsules, beads and floaters [41]. It is especially useful for dosage forms that tend to float or disintegrate slowly, for example capsules and powders because these dosage forms can be held inside the basket [43].

In USP Apparatus 2, a paddle replaces the basket as the source of agitation. The assembly is similar to the basket apparatus, except for the source of stirring. Similarly, to the USP Apparatus 1, the vessel is partially immersed in a suitable water-bath of any convenient size or heated by a suitable device such as a heating jacket at the physiological temperature. This apparatus can be used in dissolution of tablets, capsules, suspensions or powders. However, floating dosage forms require the use of sinkers.

In both apparatuses, aliquots are taken with a syringe at defined time points. Then, they are filtered through a low pore size filter (<0.45 μm) and analyzed. These systems can accommodate up to 1000 mL of medium using the standard size vessel. A medium volume of 200 to 300 ml is used to simulate the fasted stomach, 500 mL for the fasted small intestine, and up to 1000 ml for fed state conditions in the stomach and small intestine [18]. There are also non compendial smaller vessels (such as the mini-paddle) used to simulate, e.g., the fasted stomach. This mini-paddle is hydrodynamically similar to the paddle apparatus [43]. Note, however, that a reliable simulation of the GI transit is hindered due to the complexity of the hydrodynamic mechanisms of transport. For example, it is known *a priori* that the statistically steady flow is a crude approximation of the pulsated flow in the GI tract. Subsequently, several attempts to correlate the *in vitro* hydrodynamics with *in vivo* showed inconsistent results [44].

In order to simulate transfer from the stomach to the intestine, a change of media during dissolution is recommended by the pharmacopeias (commonly referred to as pH shift). This is especially relevant for modified-release forms (e.g. enteric coating formulations) or for the assessment of precipitation of APIs upon the entry in the small intestine. The pharmacopoeias recommend two different methods for changing the pH during a dissolution test in a USP Apparatus 1 and 2, one performed in a single vessel and the other including the transfer of the dosage form from a vessel containing an acidic medium simulating the gastric fluid, to a vessel containing a medium simulating the intestinal fluid. The latter mentioned method, can be performed with biorelevant media and has recently been used by Kambayashy (which they denoted as the “dumping” method) to predict the precipitation of weak basic drugs in the intestine [45].

1.3.2.3. Transfer model

The transfer model (Figure 1.5) [46] is an upgraded version of the “dumping” method. It consists of a donor compartment (e.g. mini paddle vessel) simulating the stomach and an acceptor compartment (e.g. USP Apparatus 2 vessel) simulating the intestine. Gastric emptying is mimicked through the use of a peristaltic pump which connects the donor compartment with the acceptor compartment. The pump can be programmed to run nonlinear transfer kinetics, which enables the simulation of first order gastric emptying in the fasted state. In this methodology, the biorelevant medium simulating the stomach fluid is poured in the donor vessel and the medium simulating the intestinal fluid is poured in the acceptor vessel. The media are warmed up to 37° C and the paddle rotation is turned on. Then the formulation is placed in the donor compartment. The peristaltic pump is turned on to transfer the contents of the donor compartment into the acceptor compartment and samples are withdrawn similarly to the USP Apparatus 1 and 2 assays.

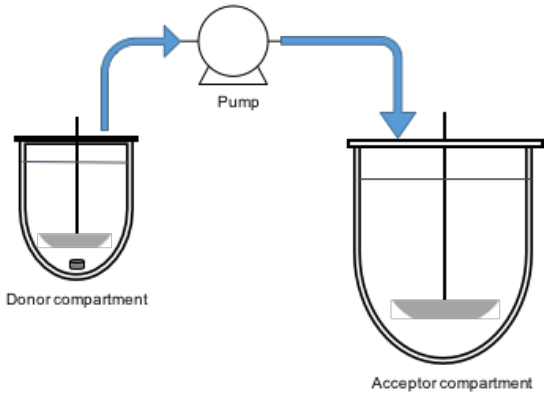


Figure 1.5 – Transfer model [46].

1.3.2.4. USP Apparatus 3 – Reciprocating cylinder

USP Apparatus 3 (Table 2.5) was incorporated into the USP in 1991 as alternative to USP apparatuses 1 and 2 for the assessment of dissolution of solid oral modified-release dosage forms [47], [48]. Its assembly consists of a set of cylindrical, flat-bottomed glass outer vessels and a set of reciprocating cylinders.

The dosage form is placed in an open cylinder fitted with a sieve at the bottom end and at the top end (optional). It is placed in a vessel heated at 37ºC and the cylinder is moved up and down through the medium. It is possible to move the cylinder from one vessel to another. If each vessel is filled with different media, it enables the simulation of the passage of the immediate and modified release drug products through the GI tract in a single experiment [44]. It allows automated testing for up to six days although media evaporation can occur for the longer duration tests.

Analogously to the USP Apparatus 1 and 2, the aliquots are withdrawn from the acceptor vessel with a syringe at defined time points, filtered through a low pore size filter (<0.45 μm) and analyzed.

The vessel capacity is low (maximum 250 mL), thus it is difficult to obtain sink conditions (i.e. a volume at least three times bigger than the one needed to obtain a saturated solution) for the case of poorly soluble drugs, although it is possible to increase the total volume in the experiment by increasing the number of times the cylinder is moved across the vessels [44]. The reciprocating cylinder comprises 6 vessels with up to 8 rows per compartment so that up to 48 vessels can be placed in it.

The reciprocating movement of the cylinder generates pulsating hydrodynamic patterns which bear some resemblance to the *in vivo* conditions, whereas the resistance to the fluid flow can be adjusted by changing the mesh size of the sieve in the bottom of the cylinder [44].

In order to mimic *in vivo* conditions it is common to use 5-15 dips per minute (dpm) to simulate the fasted state and 30-40 dpm for the fed state [47]. Moreover, it is possible to add inert

spheres of several densities in order to simulate interaction with solid food particles in movement [49].

This apparatus is originally used for extended release products and bead-type modified release dosage form, particularly beads in capsules. It is also useful for solids which are mostly non-disintegrating [43].

1.3.2.5. USP Apparatus 4 – Flow-through Cell

In 1981, International Pharmaceutical Federation proposed the flow-through cell (Table 1.5) as an alternative to basket and paddle methods for poorly soluble and extended release forms [50]. In 1990, it was accepted by the USP, becoming an official compendial apparatus [21].

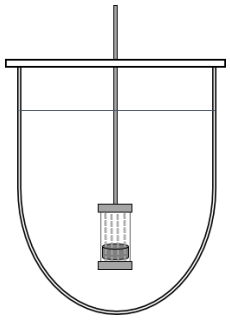
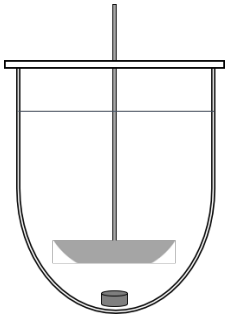
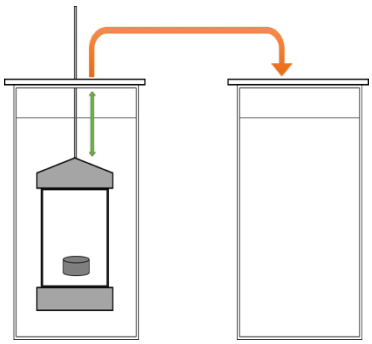
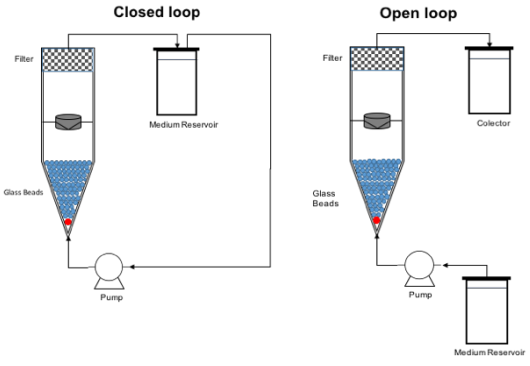
The apparatus consists of a reservoir containing a dissolution medium, a pump that forces the medium upwards through the vertically positioned flow-through cell, and a water bath that maintains the dissolution medium at 37ºC. The cell, made of a transparent and inert material, is placed vertically with a filter system that prevents the escape of undissolved particles from the top of the cell. There are two types of cells available for orally administered dosage forms: a large cell (22.6 mm i.d.) and a small cell (12 mm i.d.) The bottom cone of the cell is filled with small glass beads of about 1 mm diameter and a ruby bead of about 5 mm diameter is placed at the bottom of the cell acting as a check valve and prevents glass beads from blocking the cell channel. A filter (often a glass fiber filter) is positioned at the inner top of the cell. The filter only allows the dissolved particles to pass through and maintains the undissolved particles within the cell. Normally, single or combination filters of different pore sizes are used to optimize the filtration. Samples are usually withdrawn via an internal sampling and filtration device. In order to maintain the temperature at 37±0.5 ºC, the cells are immersed in a water bath. For other types of dosage forms, such as suppositories, powders, implants and drug eluting stents, different cell types are available. The flow through apparatus can operate in two different configurations: open system, with constant fresh medium from the reservoir passing through the cell, or as a closed system, with a fixed volume of medium passing through the cell. The pump usually has a flow rate delivery capacity between 2 and 32 mL/min, however, the compendial flowrates are 4, 8 and 16 mL/min

In the open configuration, with an exchange of media and flowrate is possible to simulate different environments of the digestive tract [51], [52]. Media volume is not limited which allows the maintenance of sink conditions, in contrast to the previous apparatuses. Therefore, it represents another approach for the investigation of modified release formulations under biorelevant conditions. Another advantage of using Apparatus 4, is that the same apparatus may be maintained through dissolution testing of all the stages of drug development, from the API to the final dosage form.

Amongst the compendial apparatuses, the flow through cell provides the most physiological hydrodynamic conditions [52]. The flow pattern is described as being turbulent when operated without glass beads in the entry cone and laminar when glass beads are used [50]. *In vivo*, in fasted and fed conditions, the average axial velocity is around 1.5 and 1.3 cm/min, respectively. In USP Apparatus 4, this corresponds to flow rates less than 8 mL/min [53].

The USP apparatus 4 is commonly used for the analysis of modified release dosage forms. The different cell types allow the testing of various drug products such as solids (tablets, capsules, implants, powders, and granules), semisolids (suppositories, soft gelatin capsules, ointments) and liquids (suspensions) [54]. Additionally, it is suitable for special dosage forms such as powders, granules and implants [41]. The flow-through cell has not been routinely used for immediate-release products, mainly because it is a complex and expensive method due to the large volumes of medium necessary. It is essentially used when the performance of the paddle and basket apparatus is insufficient. This method has been found to be superior to the paddle apparatus in achieving IVIVCs for several formulations [55]–[57].

Table 1.5 – Compendial Apparatus used for oral solid dosage forms dissolution tests [54].

USP Apparatus 1 – Basket • Typical rotating speed: 50-100 rpm • Vessel volume: 1 L • Dosage form is placed in the basket • Used for: solid dosage forms (floating, disintegrating or non-disintegrating, single units, multiple units)	
USP Apparatus 2 – Paddle • Typical rotating speed: 50-100 rpm • Vessel capacity: 1 L • Dosage form should remain at the bottom centre of the vessel • Used for: solid dosage forms (tablets, capsules), particulates (suspensions, powders) – sinkers can be used	
USP Apparatus 3 – Reciprocating Cylinder • Typical reciprocating agitation: 5-35 dps • Vessel capacity: 325 mL • Dosage form is placed in the cylinder and it moves horizontally to different rows of vessels • Used for: solid dosage forms (mostly non-disintegrating, single units, multiple units)	
USP Apparatus 4 – Flow Through cell • Operation system: open/closed • Flow rates: 2-32 mL/min (compendial: 4,8,16 mL/min) • Dosage form is placed inside the vessel, immobilized • Used for: solids (tablets, capsules, implants, powder, granules), semisolids (suppositories, soft gelatin capsules, ointments), liquids (suspensions)	

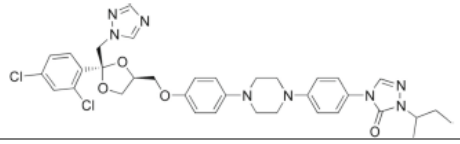
1.4. Itraconazole

The model drug used in this study, ITZ, consists in an orally active synthetic anti-fungal agent with a broad spectrum activity. It belongs to triazole group indicated in the treatment of local and systemic fungal infections (histoplasmosis, bastomycosis and onychomycosis). Its mechanism of action consists in cytochrome P-450 inhibition which prevents the synthesis of ergosterol in fungal cell membranes resulting in the membrane fluidity alteration leading to cell death [58].

It is a weakly basic (physicochemical properties in Table 1.6) and very lipophilic drug, possessing an extremely low aqueous solubility and good permeability. Because of its poor aqueous solubility, its oral bioavailability is poor. However, because of its highly lipophilic nature, ITZ can easily penetrate into the intestinal membrane. This indicates the poor aqueous solubility as the main reason for lower plasma concentrations, meaning ITZ is BCS class II and DCS class IIb.

ITZ is considered one of the most demanding challenges in the development of basic drugs due to its extremely low aqueous solubility and dissolution rate, low pKa and high dose [59].

Table 1.6 – ITZ Physicochemical Properties [59].

ITZ Physicochemical Properties	
Chemical Structure	
Aqueous solubility	~4 ng/ml
LogP	5.7
pKa	3.7
MW	705.64 (Da)
Melting Point	168 ºC

Several types of commercial brand ITZ products are available (Table 1.7). For example, Sporanox® 100 mg oral capsules, consists of an amorphous solid dispersion of ITZ in HPMC HydroxyPropylMethyl Cellulose (HPMC) coated onto inert sugar spheres and PolyEthyleneGlycol (PEG) 20.000 (to prevent the sticking of the spheres) in a hard gelatin capsule [60]. A dose between 100 and 400 mg/day is usually administered to patients.

Table 1.7 – Main marketed Itraconazole Drugs

Brand	Type	Company	Approach
Hyphanox (USA) · Onmel (Europe, Japan)	Oral Tablet	Janssen Pharmaceutica Products, LP	Amorphous Solid Dispersion (Hot Melt Extrusion)
Sporanox	Oral Capsules	Janssen Pharmaceutica NV · Janssen Pharmaceuticals, Inc.	Amorphous Solid Dispersion (Spray Drying)
Sporanox Injection	Injection Solution	Janssen Pharmaceutica Products, LP	Amorphous Solid Dispersion (HPMC)
Sporanox Oral Solution	Oral Solution	Janssen Pharmaceutica Products, LP	Cyclodextrin
Lozanoc	Oral Capsule	Mayne Pharma Group Ltd.	Amorphous Solid Dispersion, enteric coating (Spray Drying)

The absolute bioavailability of oral capsules of ITZ is 55% ± 15% [58]. ITZ should be administered with food since the bioavailability is reduced by 40% when it is administered under fasting conditions. This increase in systemic absorption in the fed state is explained by the solubility increase caused by the presence of food [61].

Also, absorption of ITZ capsules is reduced in subjects with reduced gastric acidity, such as subjects taking drugs known as gastric acid secretion suppressors (e.g., H_2-receptor antagonists, proton pump inhibitors) or subjects with achlorhydria caused by certain diseases which is consistent with the fact of ITZ being a weak base

Sporanox® capsules were used as a reference in drug product dissolution studies.

1.5. Amorphous Solid Dispersions (ASDs)

Amorphization is an approach wherein the solid state form of the drug is changed from crystalline to amorphous due to the higher solubility and faster dissolution rates characteristic of this form. However, the Gibbs free energy of amorphous forms also result in their instability and consequently, their tendency to crystallize. ASDs can be considered as a potential solution to this issue. An ASD is an amorphous molecular dispersion of a drug in a polymer matrix. The polymer carrier stabilizes the drug not only in the solid state, avoiding drug recrystallization by reducing its molecular mobility and increasing its glass transition temperature (Tg), but also offer benefits for dissolution performance by extending supersaturation [62]. Therefore, the ideal polymer must maintain the drug's amorphous form in the solid state and to dissolve quickly and release the drug in its site of absorption in the GI tract, maintaining the supersaturation for a time period such that it enables its absorption [63].

They can be formed using a variety of technologies including spray drying and hot melt extrusion. When prepared by spray drying, ASDs are designed by spray dried dispersions

(SDDs). SDDs are obtained by dissolving drug and polymer in a suitable solvent and then spray-drying the solution.

The performance of an ASD can be measured *in vitro* by its dissolution rate. The "spring and parachute" analogy (Figure 2.3) is often used to illustrate the dissolution of an ASD: The spring, a high energy form of the drug provides the driving force to solubilize the drug at a concentration greater than its equilibrium solubility level. The parachute, the carrier (or a combination of excipients) inhibits and/or retards precipitation [64].

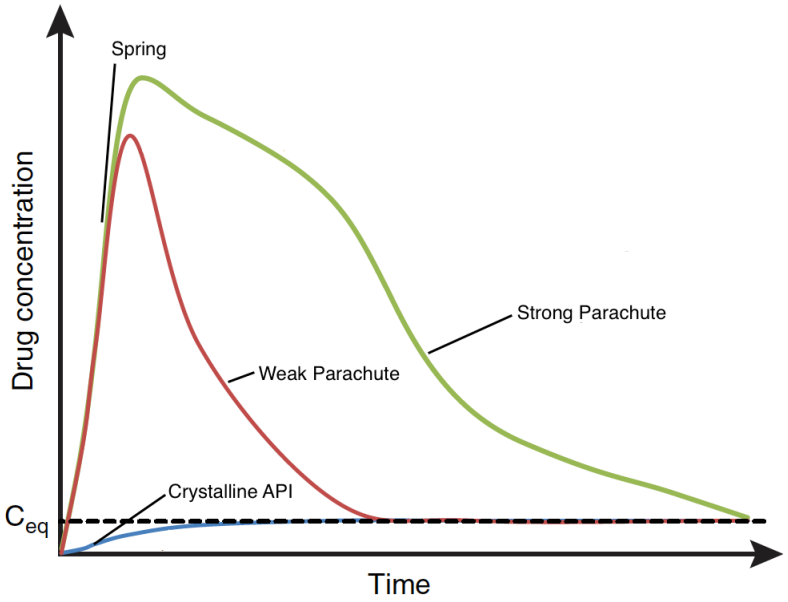


Figure 1.6 – Representation of the supersaturated state: the "spring" and "parachute" effect [71].

When added to an aqueous solution simulating the intestinal fluid, ASDs produce a variety of species, as stated by Friesen et al. [64]: free or solvated drug, drug in bile-salt micelles, free or solvated polymer, polymer colloids, amorphous drug-polymer nanostructures (20 to 100 nm), small aggregates of amorphous drug/polymer nanostructures (70 to 300 nm) and large amorphous particles (>500 nm) all of which are in dynamic exchange with each other. Several studies studies have shown that colloidal solubilized drug may not be able to permeate through the intestinal epithelium [65]–[67], highlighting the importance of knowing the "true supersaturation", i.e., the molecularly dissolved drug concentration. Since these nanoparticles cannot be separated from the solution by filtration or centrifugation, the apparent solubility of a drug can be falsely high. These drug-polymer nanostructures and nanoaggregated are believed to be critical to drug absorption since they produce a higher solubility comparing to crystalline drug and maintain the free drug concentration during drug absorption. Also, they inhibit the conversion of the amorphous drug to its crystalline form, i.e. they sustain free drug supersaturation for a physiologically relevant period of time [62], [66]–[68]. The molecularly dissolved species can be separated from the remaining species by dialysis or by ultracentrifugation [68], [69].

1.6. Main Objectives and Dissertation Outline

Taking into account the problem statement described in the introduction, the main goal of this work is the study and development of *in vitro* biorelevant dissolution methodologies that best suit the dissolution characterization of a poorly soluble drug, covering the drug substance to the final dosage form. Also, an *in silico* model will be developed, which can be calibrated using the obtained i*n vitro* data, describing dissolution phenomena of amorphous API's.

In order to accomplish the proposed goals, this document is structured in four chapters.

Chapter 1 – In the Introduction, the motivation of this work is highlighted and an overview of the most relevant concepts and methodologies available in literature regarding this subject are presented.

Chapter 2 – In the Materials and Methods a description of the used materials is given, followed by the methods used for the experimental work of this thesis, such as biorelevant media, dialysis membranes, SDDs and tablet preparation as well as the respective characterization and API quantification techniques.

Chapter 3 – In the Results and Discussion experimental results are presented regarding the solid state characterization of the formulations, dissolution and dialysis experiments and of the *in silico* model implementation and its discussion.

Chapter 4 – The Conclusions and Future Work contain the final thoughts, describing the impact of the developed work and address the direction for future research works.

Finally, the Appendix includes supplementary information regarding this work.

Part of this work was submitted and approved as a short paper to the 2nd European Conference on Pharmaceutics (Poland, 2017).

2

Materials and Methods

In this chapter, a description of the materials, procedures, and equipment used in the development/implementation of this thesis is presented. First, the methodologies regarding the production and physicochemical characterization of the ITZ, SDDs and tablets are described. Second, API solubility and dissolution studies, and third, the quantification of free drug within the ASDs and tablets with dialysis membranes.

2.1. Materials

Crystalline ITZ was supplied by Capot Chemical Co., Ltd. (Hangzhou, China). Sporanox® (Janssen Pharmaceutica, Belgium) was purchased at a local pharmacy. Biorelevant media, FaSSIF-V2 and FaSSIF/FeSSIF/FaSSGF powder were purchased from biorelevant.com (Croydon, Surrey, UK).

The polymers for SDDs production consisted of PVP/VA - Copovidone K28 (BASF, Ludwigshafen am Rhein, Germany) and hydroxypropylmethylcellulose acetate succinate (HPMCAS grade LF, AQOAT®, Shin-Etsu Chemical Co., Ltd., Tokyo, Japan).The excipients used in tablet formulation consisted of magnesium stearate (Merck, Darmstadt, Germany), croscarmellose sodium - DISOLCEL® (MINGTAI CHEMICAL CO., Ltd., Taoyuan Hsien, Taiwan), microcrystalline cellulose (Blanver, Brazil), fumed silica - Aerosil® 200 Pharma, (Evonik industries AG, Hanau, Germany), methylcellulose - methocel A15 Premium LV (Dow, Michigan, EUA) and lactose monohydrate - Tablettose® 80 (MEGGLE, Wasserburg, Munich).

FaSSGF, FaSSIF, FaSSIF-V2 and FeSSIF were prepared according to manufacturer's instructions (biorelevant.com, Croydon, Surrey, UK). FeSSGF was prepared by dissolving 13.85 g of sodium chloride, 2.445 g of sodium acetate in 0.5 L of water, 1 mL of acetic acid was then added and the mixture was diluted to 1 L with milk. Blank FaSSGF, Blank FeSSGF, Blank FaSSIF, Blank FeSSIF, Blank FaSSIF-V2, corresponding to their simple buffers and compendial media, SGFsp (Simulated Gastric Fluid sine pepsin), SIFsp (Simulated Intestinal Fluid sine pepsin), HCl 1.6 and PBS buffers 50 mM with different pHs (5.8, 6.5 and 7.2) were also prepared.

2.2. SDD and Tablet Preparation

Spray-Drying

Two formulations of SDDs were produced at 40 wt.% ITZ load with HPMCAS LF and with PVP/VA K28 - Copovidone. The drug load and spray drying process conditions mimicked those employed in a previous work [70]. Solutions of ITZ and each polymer were prepared with 10 wt.% solids concentration in dicloromethane (DCM) for the case of PVP/VA and a mixture of DCM and methanol (MeOH) (80:20 w/w) for HPMCAS LF. SDDs were produced in a laboratory scale spray dryer (BÜCHI Mini Spray Drier B-290, Switzerland) equipped with a two fluid nozzle with a 0.7 mm orifice and 1.4 mm cap. In both runs, the spray drying unit was operated in open cycle mode, i.e. without recirculation of the hot drying gas (nitrogen). The drying gas fan was set at 100% of its capacity (flow rate at maximum capacity corresponds approximately to about 40 kg/h). A flow rate of 0.76 kg/h was set for the atomization with nitrogen. Before feeding the solution to the nozzle, the spray dryer was stabilized with nitrogen and then with a solvent mixture without solids to ensure stable inlet and outlet temperatures (T_{in} and $T_{out,}$ respectively). The feed flow rate was set to 30% in the peristaltic pump (about 11.5 mL/min of liquid feed). The inlet temperature was adjusted to achieve an outlet temperature of 40°C for both trials. The stream containing the dried particles was directed into a cyclone and collected at the bottom of the cyclone. After spray-drying, SDDs were post-dried step in a tray dryer with a temperature of 40°C for approximately 12 h, under vacuum in order to remove any residual solvents. The amorphous state and single phase of SDDs was confirmed by X-ray powder diffraction (XRPD) and Differential Scanning Calorimetry (DSC) analysis and its stability by repeating the XRPD analysis after two months of storage at room temperature.

Tableting

Tablets containing the previously prepared SDDs (100 mg of ITZ) were prepared as per the formulations indicated in Table 2.1. The intergranular excipients were mixed in a TURBULA® - T2F (Willy A. Bachofen AG Maschinenfabrik, Switzerland). All the intragranular excipients were blended for 15 minutes at 46 rpm and then magnesium stearate was added and mixed for 2 min at 32 rpm. The blend of the powders was compressed into tablets on a PMS ST8 Mpress R&D Tablet Press (PMS, Surrey, UK), but using solely one punch and operating manually due to the low quantities being processed, which comes at the cost of lesser control of the dwell time (i.e. duration of the compression process), which is nevertheless secondary to the objective of the current work. For every tablet, 500 mg of the mixture were filled into the die and compressed to a hardness between 10 and 15 kP by controlling the tablet edge thickness (minimum distance between the punches during the compression process). The hardness of the first 5 tablets was verified for quality control purposes with a Sotax HT 1 Hardness tester (Sotax, Switzerland). Then, the tablets were granulated through a mesh no. 28 (roughly 600 μm aperture), except for formulation 2 where the powder was divided in three equal parts and granulated through sieves with

different apertures: 212, 600 and 800 μm. The disintegrant (croscarmellose sodium) was only added before the granulation so that the drug would be released from granules of known size. The extragranular excipients - lactose, microcrystalline celullose (MCC), croscarmellose sodium and magnesium stearate - were added, blended one more time and the powder was weighted (500 mg per tablet) and compressed into tablets again. The amorphous form of ITZ within the tablets was confirmed by DSC and XRPD analysis.

Table 2.1 – Formulation Blend Composition.

		Formulation 1	Formulation 2	Formulation 3	Formulation 4
SDD formulation/binder used		PVPVA SDD – MCC Binder	PVPVA SDD - HPMC Binder	HPMCAS SDD - MCC Binder	HPMCAS SDD - HPMC Binder
%Intragranular	SDD ITZ	50.0	50.0	50.0	50.0
	HPMC	-	16.5	-	16.5
	MCC	16.5	-	16.5	-
	Lactose	15.5	15.5	15.5	15.5
	Fumed Silica	0.7	0.7	0.7	0.7
	Magnesium Stearate	0.4	0.4	0.4	0.4
%Extragranular	Lactose	6.5	6.5	6.5	6.5
	MCC	9.5	9.5	9.5	9.5
	Croscarmellose Sodium	0.4	0.4	0.4	0.4
	Magnesium Stearate	0.5	0.5	0.5	0.5
Drug Load (%)		20.0	20.0	20.0	20.0

2.3. SDD and Granule Solid State Characterization

XRPD

Diffraction patterns were obtained with a PANalytical X'Pert PRO X-ray Diffractometer (PANalytical, Almelo, Netherlands) in alpha1 configuration equipped with an X'Celerator detector. Cu (λ = 1.5406 Å) was used as anode material and crystal graphite monochromator, operated at a voltage of 40 kV and a current of 45 mA. Samples were pressed into a sample holder to generate a flat and smooth plane surface and were analysed in the 2θ scan range of 3.0000-39.9987°. The process parameters were set as follows: step size of 0.0167° (2θ), scan step time of 59.690 seconds, and time of acquisition of 18 minutes and 29 seconds.

DSC

Conventional and modulated DSC (mDSC) experiments were performed in a DSC Q200 V24.4 Build 116 (Universal V4.5A TA Instruments, USA) equipped with a refrigerated cooling system (RCS), after adequate calibration with indium (TA instruments, USA; T_{melt} =156.55°C, $\Delta_{melt}H$ = 28. 51 J/g).

Samples, weighing between 3 and 5 mg were placed in pin-holed aluminium pans (Perkin-Elmer, Massachusetts, EUA). In the case of conventional DSC, samples were equilibrated at 20ºC and analyzed under continuous dry nitrogen purge (50 mL/min) in a ramp of 10 ºC/min until 300ºC. In the case of mDSC, samples were equilibrated at 0ºC and analyzed using a modulated heating ramp from that temperature to 250°C at a heating rate of 2.5°C/min using a period of 60s and an amplitude of 1.2°C. In order to obtain ITZ glass transition (Tg), the product was subjected to a heat-cool-heat cycle (conventional DSC) to render the amorphous product and then, the modulation cycle was applied. The sample was equilibrated at 0ºC, and heated until 250ºC at 10ºC/min. Then, it was cooled at 50º C/min until 0ºC. Afterward, the mDSC method previously stated was applied. Data was processed using the TA Universal Analysis 2000 Software (Waters, Milford, MA, USA). Tg was considered as the inflection point in the heat capacity change (ΔCp) observed in the reversible heat flow.

SEM (Scanning Electron Microscopy)

Samples were attached to adhesive carbon tapes (Ted Pella Inc., CA, USA), previously fixed to aluminium stubs and the powder in excess was removed by a jet of pressurized nitrogen. A Phenom ProX (Phenom-World BV, Eindhoven, Netherlands) scanning electron microscope operated at an accelerating voltage of 10 kV was employed. Micrographs were taken at various magnifications, ranging from 270x to 4900x.

PSD (Particle Size Distribution) – Analytical Sieving

To perform dissolution experiments, tablets were sieved by a mesh corresponding to their particle size. To assess the granule size distribution, granules obtained were fractionated into six different sizes (750, 500, 355, 250, 125 and 45 μm) by shaking for 10 min with an interval of 30 s and an amplitude of 1.5 with a nest of sieves mounted on a test sieve shaker HAVER EML 450 Digital Plus (HAVER & BOECKER, Oelde, Germany). Granule size distribution was determined by the weight of granules in each sieve. The d50, the median granule size, is taken as the sieve mesh size for which half the sample (by weight) can pass.

PSD – Laser Diffraction

The particle size of the spray dried amorphous solid dispersion of ITZ was measured by laser diffraction (SYMPATEC, Sympatec Inc., Pennington, NJ, USA). The instrument is equipped with a dispensing unit RODOS/M, a vibratory feeder VIBRI, and a laser diffraction sensor HELOS. Dry powders were measured directly with a dispersing pressure of 3 bar at a feed velocity of 18

mm/s and a laser measuring range of 0.5 µm to 350 µm. The measurements were performed in duplicate.

2.4. HPLC (High Performance Liquid Chromatography)

The quantification of ITZ throughout the *in vitro* studies was conducted by Ultra Performance Liquid Chromatography (UPLC), a variant of HPLC technique employing higher pressures, using a Waters Acquity UPLC H-Class chromatograph with an UPLC® Acquity PDA detector system (Waters, Milford, MA, USA). 1 µL of sample solution equilibrated at 25 ºC was injected onto an Acquity UPLC CSH C18 2.1 mm x 100 mm, 1.7 µm (Waters, Milford, MA, USA) held at 40 ºC and eluted with a mixture of acetonitrile, tetrahydrofuran (THF) and water adjusted to pH 2.5 with phosphoric acid (50:2.5:47.5) v/v, respectively, at a flow rate of 0.4 mL min^{-1}. The effluent was monitored at a UV wavelength of 254 nm. Mobile phases and washing solutions were degassed and sonicated in a USC-THD (VWR, USA) for 5 min before the analysis. The chromatograms were acquired and processed using Empower™ 3 Chromatography Data Software (Waters, Milford, MA, USA). The linearity of this method was demonstrated by a calibration curve, in triplicate (n=3), of ITZ in the dissolution mixture (MeOH:H_2O 90:10 v/v) between 1 and 75 µg/mL (Appendix 1). ITZ maximum absorption wavelength was confirmed in a mixture of H_2O:MeOH (90:10 v/v) using a HITACHI U-2000 UV Spectrophotometer (HITACHI, Ltd., Tokyo, Japan).

2.5. Drug content in SDDs and granules

SDDs

The drug content in the solid dispersions was assayed according to the HPLC method described before. Concentrated stock solutions of the respective solid dispersions in MeOH were prepared by dissolving 12.5 mg of the SDDs in 25 mL of MeOH in a 50 mL volumetric flask. The solutions were vortexed, sonicated for 5 min in an Ultrasonic Cleaner USC-THD (VWR, USA) and shaken for 30 min in a CAT S50 Microplate shaker (CAT, Staufen, Etzenbach, Germany). After resting, the volume was completed with MeOH. Standard solutions with a target ITZ concentration of 50 µg/mL were prepared by diluting 10 mL of each concentrated stock solution with MeOH:H_2O 90:10 v/v in a 20 mL volumetric flask. The solutions were sonicated prior to analysis, filtered through GHP Acrodisc® 25 mm syringe filters, with 0.45 µm GHP Membrane (Pall Life Sciences, Port Washington, New York, Pall) and injected. The quantification was performed against a single-point external standard of pure ITZ in MeOH:H_2O 90:10 v/v (50 µg/mL). This analysis was carried out in duplicate (n=2).

SPO and granules

The assay content of the granules was performed according to the HPLC method described before. In a 200 mL volumetric flask, 100 mg of granules were submitted to sonication with 20 mL

of H_2O, for 10 minutes on an Ultrasonic Cleaner USC-THD (VWR, USA). About 100 mL of MeOH was added to the volumetric flask and vortexed for 1 min. After resting, the volume was completed with MeOH. This solution was diluted 1:1 with a mixture of MeOH:H_2O (90:10, v/v) and then filtered through GHP Acrodisc® 25 mm syringe filters, with 0.45 µm GHP Membrane (Pall Life Sciences, Port Washington, New York, Pall). The quantification was performed against a single-point external standard of pure ITZ in MeOH:H_2O 90:10 v/v (50 µg/mL). This analysis was carried out in duplicate (n=2).

2.6. API Solubility and Dissolution Studies

Thermodynamic Solubility of the API

ITZ saturation solubility was determined using the Shake Flask method [2]. An excess of drug (approximately 10 mg) was added to 20 mL scintillation vials containing 10 mL of the dissolution media (miliQ water, SGFsp, HCl 1.6, SIFsp (EP 8), PBS buffers 50 mM with different pHs - 5.8, 6.5 and 7.2, FaSSGF, FaSSIF, FeSSGF, FeSSIF, FaSSIF (and respective blanks, corresponding to the buffer without solubilizers), vortexed and then placed on an ES – 20/60, Orbital Shaker – Incubator (Biosan, Latvia) for 24 h at 37±0.5 ºC and 50 rpm. After this period, the vials were vortexed. The suspension was centrifuged in a himac CT15RE centrifuge (Hitachi Koki Co., Ltd.) for 3 min at 13000 rpm at room temperature. The supernatant was filtered through Bulk IC Acrodisc® 13 mm, 0.45 µm Supor® (PES) syringe filters (Pall Life Sciences, Port Washington, New York, Pall). Regarding the milk-based medium, FeSSGF, an additional step was employed to allow the precipitation of protein proteins, thus enabling a proper quantification. An aliquot of 1 mL was withdrawn in the middle of the flask. Then, 1 mL of MeOH was added. After this, the sample was centrifuged in a himac CT15RE centrifuge (Hitachi Koki Co., Ltd.) for 3 min at 13000 rpm at room temperature. These tests were carried out in triplicate (n=3). As the chromatographic method was proven to be linear (see Appendix 1), the quantification was performed against a single-point external standard of pure ITZ in MeOH:H_2O 90:10 v/v (50 µg/mL).

Intrinsic Dissolution Rate (IDR)

IDR testing of ITZ was performed using a miniaturized method as surrogate of the USP Rotating Disk Apparatus. 100 ± 5 mg of ITZ were compressed for 1 min in a diameter die against a steel plate under a pressure of 9000 ton using a punch and die system (Atlas 15T Manual Hydraulic Press, SPECAC Ltd., Kent, UK) to yield a disk of known surface area. The disks were blown with compressed air to remove any loose particles and their dimensions measured with a caliper. Each pellet, was then placed on the top of a cavity of a 6-well-plate containing 10 mL of the dissolution medium and incubated in an ES – 20/60, Orbital Shaker – Incubator (Biosan, Latvia) at 37±0.5 ºC and 50 rpm. Aliquots of 1 mL were withdrawn at 1, 5, 10, 15, 20, 30, and 60 minutes with dissolution media volume replacement. The samples were filtered through Bulk IC Acrodisc® 13 mm, 0.45 µm Supor® (PES) syringe filters (Pall Life Sciences, Port Washington,

New York, Pall) and analysed by HPLC. The media used consisted of FaSSGF, FeSSGF, FaSSIF, FaSSIF-V2 and FeSSIF. Regarding the milk-based medium, FeSSGF, 1 mL of MeOH was added to 1 mL of the aliquot in order precipitate milk proteins. After this, the sample was centrifuged in a himac CT15RE centrifuge (Hitachi Koki Co., Ltd.) for 3 min at 13000 rpm at room temperature. These tests were carried out in triplicate (n=3). The quantification was performed, by HPLC, against a single-point external standard of pure ITZ in MeOH:H_2O 90:10 v/v (50 µg/mL).

2.7. Drug Product Intermediate/ Drug Product Dissolution Studies

Dissolution

Dissolution studies of ITZ solid dispersions were conducted using the USP dissolution apparatus 2 with a Copley Dissolution Tester DIS 6000 (Copley, Nottingham, UK). 900 mL of dissolution media (either FaSSGF or FaSSIF) were added to 1 L capacity vessels containing the dosage form (corresponding to 100 mg of ITZ). The dosage forms consisted of the SDD powders, tablet granules and the content of SPO capsules. The test was carried out at 75 rpm and 37 ± 0.5 ºC. Samples of 5 mL were taken at 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240 min and then again after 15 min at 200 rpm (infinity spin) with dissolution media volume replacement. All samples were filtered immediately through GHP Acrodisc® 25 mm syringe filters, with 0.45 µm GHP Membrane (Pall Life Sciences, Port Washington, New York). The first 3 mL of the filtrate were discarded. The remaining volume was diluted with MeOH in a 1:1 v/v proportion and analyzed by HPLC. The dissolution experiments were carried out in duplicate (n=2). The non enteric formulations were tested in gastric media and the enteric formulations in the intestinal media. Also, the best formulation of tablets of each group (enteric/non enteric) were used to perform dissolution testing in FeSSIF and pH shift method using FaSSGF and FeSSIF in the fasted state. The pH of FeSSIF was adjusted to 5.8 so that the enteric polymer HPMCAS LF would dissolve. SPO was additionally tested in SGFsp, FaSSGF with its pH adjusted to 1.2 and HCl 1.6 in order to assess the pH effect in ITZ dissolution. The quantification was performed, by UPLC, against a single-point external standard of pure ITZ in MeOH:H_2O 90:10 v/v (50 µg/mL).

pH Shift Simulating Fasted State

Dissolution studies of ITZ tablets and SPO capsules were conducted using the USP dissolution apparatus above mentioned. This experiment was designed according to biorelevant methodology, with biorelevant volumes and transit times. 250 mL of gastric media (FaSSGF) were added to 1 L capacity vessels containing 500 mg of the dosage form (or the content of one capsule, in the case of SPO). The test was carried out at 75 rpm and 37 ± 0.5 ºC. Samples of 5 mL were taken at 5, 10, 15, 20, 30, 45 and 60 minutes. At this point, 250 mL of 2x concentrated FaSSIF and 5 mL of sodium hydroxide (to adjust the pH to 6.5) were added to the vessel. Aliquots

were taken at 5, 10, 15, 20, 30, 45 and 60, 90, 120 and every half hour until 5 hours and then again after 15 min at 200 rpm (infinity spin), with dissolution media volume replacement. All samples were filtered immediately through GHP Acrodisc® 25 mm syringe filters, with 0.45 µm GHP Membrane (Pall Life Sciences, Port Washington, New York, Pall). The first 3 mL of the filtrate were discarded. The remaining volume was diluted with MeOH in a 1:1 v/v proportion and analyzed by UPLC against a single-point external standard of pure ITZ in MeOH:H_2O 90:10 v/v (50 µg/mL).

2.8. Quantification of free drug within the ASDs with dialysis membranes

API Dissolution & Permeability Test

A dissolution and permeability test of the API was performed with 100 mL intestinal medium (FeSSIF) containing a dialysis membrane – Float-A-Lyzer®G2 Dialysis Device with a molecular weight cutoff of 1000 kD (Spectra/Por®, USA). The membranes were prepared according to manufacturer's instructions [71]. In a 250 mL goblet, the dissolution media was allowed to equilibrate at 37ºC; 100 mg of an ITZ IV pellet or powder were placed in each goblet. At time points 1, 5,10,15, 20, 30, 60, 90 and 24h, 1 mL of media were aliquoted inside and outside the membrane – this method is known as reverse dialysis. The volume was replaced and the samples from outside the membrane were filtered through Bulk IC Acrodisc® 13 mm, 0.45 µm Supor® (PES) syringe filters (Pall Life Sciences, Port Washington, New York, Pall). Samples were analyzed by HPLC. The quantification was performed against a single-point external standard of pure ITZ in MeOH:H_2O 90:10 v/v (50 µg/mL). This analysis was carried out in duplicate (n=2).

In order to assess if crystalline ITZ was able to permeate through the dialysis membrane (previously prepared according to manufacturers instructions [71]), 100 mg of ITZ were added to shot flasks containing 400 mL with dissolution medium - HCl 1.6 with 1% of sodium dodecyl sulfate (SDS) – incubated in an ES – 20/60, Orbital Shaker – Incubator (Biosan, Latvia) at 37 ± 0.5 ºC and 100 rpm with four dialysis membranes - Biotech CE (Cellulose Ester) Membrane with a molecular weight cutoff of 3.5-5 kD (Spectra/Por®, USA) containing 500 µL of dissolution medium. At each time point – 30, 60, 120, 180 minutes, an aliquot of 2 mL and one membrane was taken from outside the membrane and filtered through GHP Acrodisc® 25 mm syringe filters, with 0.45 µm GHP Membrane (Pall Life Sciences, Port Washington, New York). The filtrate was diluted 1:1 with MeOH before HPLC analysis. The content of the membrane was directly analyzed. The quantification was performed against a single-point external standard of pure ITZ in MeOH:H_2O 90:10 v/v (50 µg/mL). This analysis was carried out in duplicate (n=2).

SDD Dissolution & Permeability Tests

This experiment was conducted in USP Apparatus 2 at 75 rpm with 900 mL of dissolution media. The non enteric SDD (ITZ: PVP/VA) was tested in the fasted gastric media (FaSSGF) and the enteric SDD (ITZ:HPMCAS LF) was tested in the fasted intestinal media (FaSSIF). Six dialysis membranes containing 500 µL of dissolution media were prepared for each vessel. Membranes were previously washed according to manufacture procedure [71]. An equivalent of 100 mg of ITZ was placed in the vessel and an aliquot of 5 mL was taken at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes. The volume taken was not replaced. 500 µL of the dissolution samples were withdrawn and diluted it with 500 µL of MeOH. Before dilution, this samples were filtered through GHP Acrodisc® 25 mm syringe filters, with 0.45 µm GHP Membrane (Pall Life Sciences, Port Washington, New York, Pall) and the first 3 mL were discarded. At 5, 15, 30, 60, 90 and 120 minutes, one membrane was taken and its content directly analyzed. Samples were analyzed by HPLC. The quantification was performed against a single-point external standard of pure ITZ in MeOH:H_2O 90:10 v/v (50 µg/mL). This analysis was carried out in duplicate (n=2).

Granule Dissolution & Permeability Tests

The pH shift method was performed in USP Apparatus 2 at 75 rpm. 250 mL of the gastric media (FaSSGF) were added to 1 L vessels containing either one SPO capsule content or 500 mg of formulation 4 (enteric). Membranes were prepared according to manufacture procedure. Six dialysis membranes containing 500 µL of dissolution media were prepared for each vessel. At 60 minutes, 250 mL of 2x concentrated FaSSIF and 3 mL of 1 M NaOH were added to the vessel together with the 6 dialysis membranes. An equivalent of 100 mg of ITZ was placed in the vessel and an aliquot of 5 mL was taken at 5, 10, 15, 30, 60, 65, 75, 90, 120, 180 and 240 minutes and the volume taken was not replaced. 500 µL of the dissolution samples were withdrawn and diluted it with 500 µL of MeOH. Before dilution, this samples were filtered through GHP Acrodisc® 25 mm syringe filters, with 0.45 µm GHP Membrane (Pall Life Sciences, Port Washington, New York, Pall) and the first 3 mL were discarded. At 65, 75, 90, 120, 180 and 240 minutes, corresponding to the intestinal environment, one membrane was taken and its content directly analysed by HPLC. The quantification was performed against a single-point external standard of pure ITZ in MeOH:H_2O 90:10 v/v (50 µg/mL). This analysis was carried out in duplicate (n=2).

3

Results and Discussion

In this chapter, key experimental results obtained are presented, interpreted and discussed: the results regarding API (Active Pharmaceutical Ingredient) dissolution; SDD and tablet production and respective solid state analysis – XRPD, DSC and mDSC, SEM; SDD and tablet dissolution; dialysis membranes for free ITZ quantification studies; and prospects for modeling, where the equations of the *in silico* dissolution model implemented in Scilab and the respective output are presented.

3.1. API Dissolution Studies

The determined ITZ solubility in the media tested was below the LOQ of the method, 1 µg/mL. Only the value of thermodynamic solubility of ITZ in SGFsp was obtained, corresponding to 3.07 ± 0.3 µg/m, not too far from the one found in the literature, 3.9 ± 0.7 µg/mL [72]. Accordingly, the results of IDR and dissolution and permeation test of the API could not be obtained.

Figure 3.1 – The Shake Flask method for ITZ solubility determination. On the left, vials containg ITZ in FaSSGF and FaSSIF before incubation and on the right, the same vials after 24h of incubation, at 37ºC.

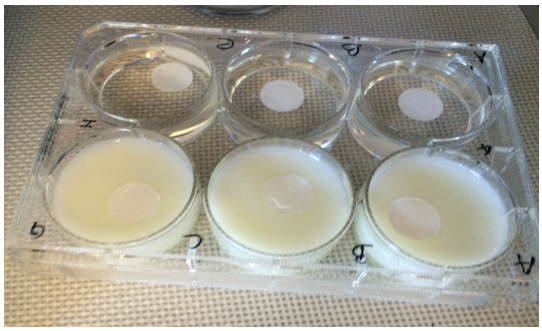


Figure 3.2 – ITZ IDR determination in FaSSGF and in FeSSGF, using a multiwell plate.

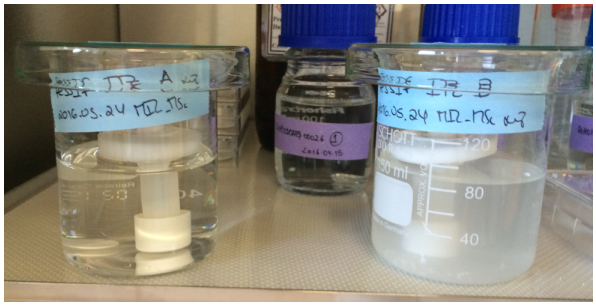


Figure 3.3 – ITZ dissolution and permeation experiment in FeSSIF. On the left, ITZ is available as a compressed pellet, on the right ITZ is available as a powder.

These tests (Figure 3.2, 3.3 and 3.4) confirmed that ITZ is a drug whose absorption is limited by its solubility, indicating that a solid dispersion is a suitable formulation approach for this API.

3.2. SDD and Tablet Preparation

SDDs

Two different types of polymer stabilizers were chosen to generate an immediate release formulation: PVP/VA in order to produce extensive supersaturation in acid and HPMCAS LF, which dissolves at a pH equal or higher than 5.5, ensuring its release only in intestinal media (enteric release). This last polymer should provide minimal ITZ release in acid but will produce extensive supersaturation following the acidic-to-neutral pH transition.

The yield obtained in the two spray drying runs was of 82% which is in-line with the previous experience at Hovione. The main properties of the ITZ powders produced are displayed in Table 3.1. Henceforth, the three resultant formulations of formulation 2 with different granule sizes will be designated by its respective sieve apertures: 212, 600 and 800 μm.

Table 3.1 – Summary of produced SDDs properties.

	SDD ITZ:PVP/VA	SDD:HPMCAS LF
Amorphous (mDSC and XRPD analisys)	Yes	Yes
Single phase (mDSC)	Yes	Yes
Tg (ºC)	91.03	86.58
Particle D50 (µm)	3.94	3.18
Particle Span	2.3	2.5
Particle Morphology (SEM analysis)	Spherical particles	Buckled particles

Figure 3.4 – SDD production in a Buchi Mini Spray Dryer B-290.

Tablets

As reported in the section 2.2 of this thesis, the tablet compression was performed in two steps. The first one, known as slugging, consists of formulating without disintegrant in order to simulate a dry granulation process (e.g. roller compaction). After this, disintegrant (and the other extragranular excipients) were added and the granules were compressed to tablets (second step). However, this strategy was not successful, since in the first dissolution experiment, it was verified that the produced tablets did not disintegrate. This was possibly due to an insufficient percentage of sodium croscarmellose formulation – 1% instead of 2-5%, the indicated range for tablets [73]. As a compromise alternative, the granules were milled again through the same sieve sizes as before, in order to reverse the compression step.

The average yield of the entire process, dry granulating and tableting, was 59.4%. This low value is expectable, due to the large mass losses that occurred during tableting process. Tablets were weighted and compressed individually (Figure 4.6). If the automatic feeder of the tablet

press had been used, even lower yields would be observed. The main properties of the ITZ tablets produced are displayed in Table 3.2.

Figure 3.5 – Six formulations of ITZ tablets produced.

Table 3.2 – Summary of produced tablet properties.

	Non enteric formulations				Enteric formulations	
Formulation	**1 - 600 μm**	**2 - 212 μm**	**2 - 600 μm**	**2 - 800 μm**	**3 - 600 μm**	**4 - 600 μm**
wt. % Excipients	**PVP/VA ASD – MCC Binder**	**PVP/VA ASD - HPMC Binder**			**HPMCAS ASD – MCC Binder**	**HPMCAS ASD - HPMC Binder**
ASD	49.7	50.2	50.0	50.2	49.6	49.6
HPMC	0.0	16.8	16.7	17.3	0.0	16.6
MCC	25.2	8.6	8.5	8.6	25.3	8.6
Lactose	21.0	21.2	21.1	21.2	23.9	21.1
Fumed Silica	0.8	0.8	0.8	0.8	0.8	0.8
Magnesium Stea-rate	0.9	0.9	1.4	0.9	0.9	0.9
Croscarmellose Sodium	1.0	1.0	1.0	1.0	1.0	1.0
Drug load	19.9	24.4	23.5	23.7	19.9	19.9
d50 mass	250 μm	45 μm	250 μm	355 μm	250 μm	250 μm
Amorphous - XRPD	Yes	Yes	Yes	Yes	Yes	Yes
Amorphous - DSC	Yes	Yes	Yes	Yes	Yes	Yes

3.3. Drug Content Within ITZ formulations

HPLC analysis was used to estimate the drug content in the formulations (Table 3.3).

Table 3.3 – ITZ content within the formulations tested.

	Average Drug Content % w/w (n=2)	% RSD (n=2)	Theoric Drug Load (%)
SDD ITZ:PVP/VA	102.5	2.6	40.0
SDD ITZ:HPMCAS LF	104.0	3.5	40.0
SPO	101.5	0.4	20.0
Formulation 1	99.8	0.1	19.9
Formulation 2 - 212 µm	101.4	0.8	20.1
Formulation 2 - 600 µm	106.4	5.4	20.0
Formulation 2 - 800 µm	108.3	0.6	20.1
Formulation 3	99.3	0.5	19.9
Formulation 4	103.2	0.6	19.9

The obtained ITZ weight percentage in the SDDs was between 99% and 101% of the theoretical value. The obtained values for the all the tablet formulations tested were between 80% and 105% (w/w) of the theoretical values. ITZ content in the commercial product, SPO, was between 101% and 102% of the label claim.

3.4. SDD and Granule Solid State Characterization

Solid state characterization was performed to confirm the amorphous state of the API contained in the formulations and that there is no phase separation (XRPD and DSC). Additionally, to evaluate if the SDDs had comparable particle sizes, PSD analysis by laser diffraction was performed. To assess the granule PSD, and thus its influence in dissolution, analytical sieving was conducted. Finally, DSC analysis was used to assess if the compression step modified the polymorphic form of ITZ. SEM analysis was used to study SDDs morphology and to confirm the results of PSD determination by laser diffraction.

Crystallinity and phase separation studies

The ITZ characterization by XRPD allowed the acquisition of the characteristic diffractogram pattern of the form I (Figure 3.6). The diffractogram pattern of Figure 3.6 is consistent with that of the commercial form polymorph [86]. Figure 3.6 also confirms the amorphous state of the SDDs produced, showing the characteristic halo of amorphous forms. It can be seen that the physical mixtures show crystallinity, corresponding to ITZ even though the intensity of the reflections is smaller than in raw ITZ, due to the drug load of 40%. Additionally, pure carrier polymers, HPMCAS and PVP/VA exhibit characteristic broad amorphous halos.

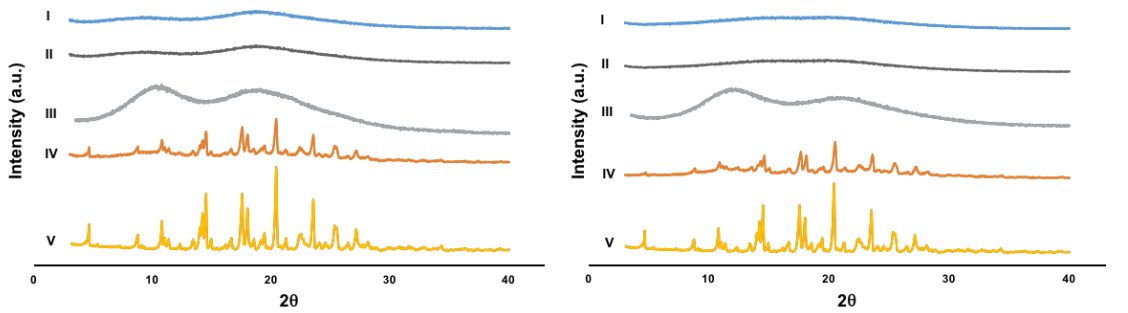


Figure 3.6 – XRPD patterns. Left: I: Fresh SDD ITZ:HPMCAS LF; II: SDD ITZ:HPMCAS LF after two months; III: HPMCAS LF; IV: SDD ITZ:HPMCAS LF Physical Mixture; V: Crystalline ITZ. Right: I: Fresh SDD ITZ:PVP/VA; II: SDD ITZ:PVP/VA after two months; III: PVP/VA; IV: SDD ITZ:PVP/VA Physical Mixture; V: Crystalline ITZ.

The physical stability of the produced dispersions was assessed by repeating the XRPD analysis after two months after their production, using samples stored at room temperature and humidity (Figure 3.6). The 2-month old samples show the same pattern of the fresh ones. This indicates that ITZ was converted to a physically stable amorphous form. The presented SDD stability is expectable, since their glass transition temperatures (Tgs) are greater than 20ºC above the storage condition, even in the summer, when higher temperatures occurred.

From Figure 3.7, it can be seen that the reflections corresponding to the higher intensity reflections of crystalline ITZ can only be seen in the physical mixture diffractograms. All the peaks presented in the formulation XRPD patterns, corresponding to crystalline excipients - MCC MgSt, lactose monohydrate, are also present in the corresponding physical mixtures, showing that there is no formation of an alternative polymorph due to the compression in the tableting step. Therefore, these diffractograms do not show ITZ crystallinity.

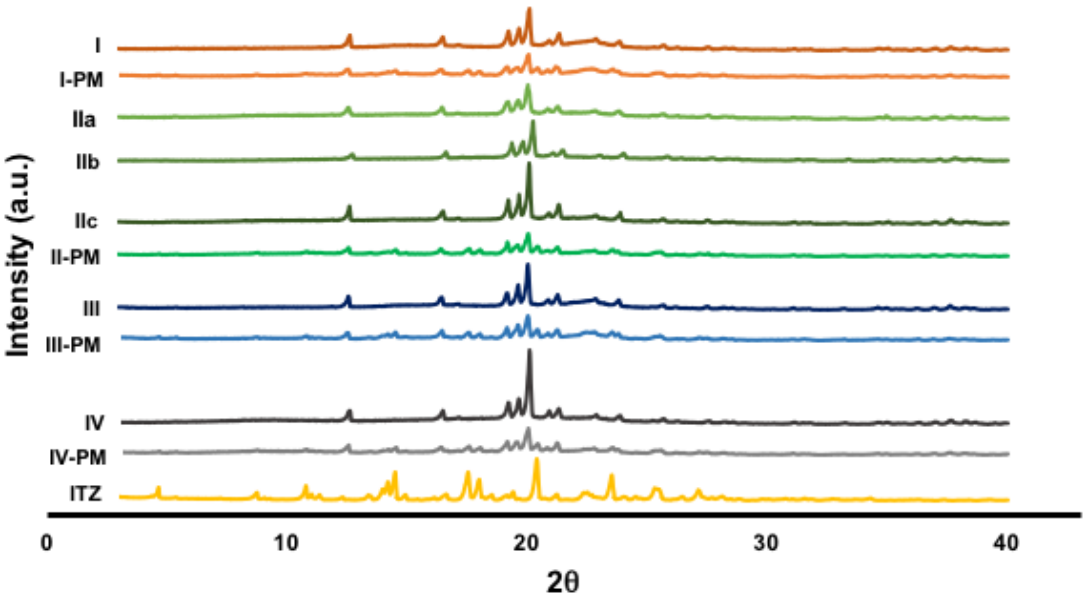


Figure 3.7 – Diffractograms of ITZ tablet formulations. I: Formulation I; I - PM: Physical mixture of Formulation 1; IIa: Formulation 2 – 212 μm; IIb - Formulation 2 – 600 μm; IIc: Formulation 2 – 800 μm; II-PM: Physical mixture of Formulation 2; III: Formulation 3; III-PM: Physical mixture of Formulation 3; IV: Formulation 4; VI–PM: Physical mixture of Formulation 4; ITZ: Crystalline ITZ.

XRPD analysis confirms the amorphous nature of the compounds, but does not provide an answer regarding the existence of phase separation, i.e., amorphous-amorphous separation within the SDDs. The answer is given by DSC analysis by the existence of a single Tg.

ITZ thermogram (Figure 3.8) shows a single, well defined, endothermic peak at 166.72°C corresponding to its melting temperature. This endotherm is characteristic of a pure substance, presenting a linear melting curve. Therefore, in agreement with XRPD analysis, the analyzed ITZ is classified as crystal form I, the most stable polymorph, with a melting temperature of 165.1 ºC [74].

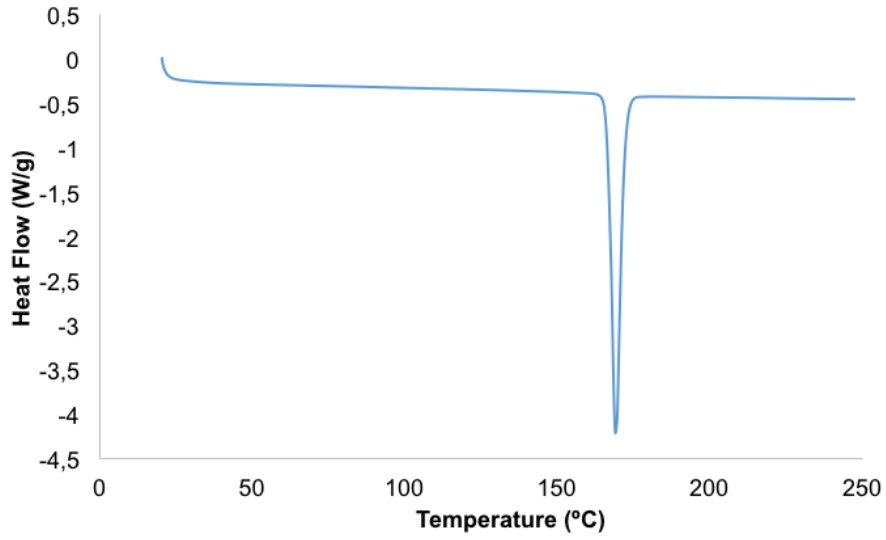


Figure 3.8 – Crystalline ITZ DSC thermogram.

PVP/VA being an amorphous polymer showed a single Tg of 113.95 ºC (Figure 3.9). The ITZ Tg was determined as 58 ºC (Figure 3.9). This value, which is relatively high, is in agreement with the literature [74]. The endotherms at 70.3 and 90 ºC result from the formation of a mesophase [75].

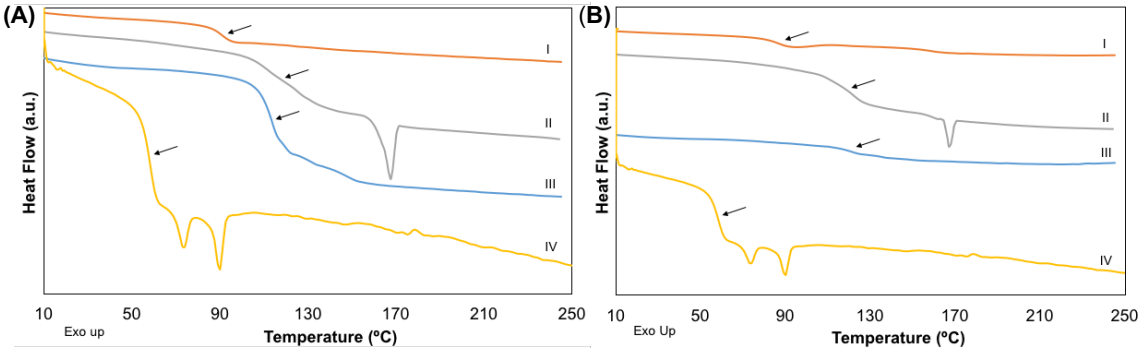


Figure 3.9 – mDSC thermograms. Left (A): I: SDD ITZ:HPMCAS LF; II: Physical Mixture of SDD ITZ:HPMCAS LF; III: HPMCAS LF; IV: Amorphous ITZ. Right (B): I: SDD ITZ:PVP/VA; II: Physical Mixture of SDD ITZ:PVP/VA; III: PVP/VA; IV: Amorphous ITZ. The arrows point to the glass transition temperatures.

Regarding the thermogram of SDD ITZ:HPMCAS LF, also an amorphous polymer, it shows a single Tg of 120.78 ºC (Figure 3.9 (A) - Left). Considering the thermogram of SDD ITZ:HPMCAS LF (Figure 3.9 (A) - Left), there is a single Tg at 86.58 ºC, indicating the presence of a single phase. The obtained Tg is an intermediate value between the ITZ and polymer. The Tg of the SDD ITZ:HPMCAS LF was estimated using the simplified Gordon-Taylor/Kelley-Bueche (GTKB) equation [76]. The obtained value was of 93ºC, higher than the experimental one. However, GTKB model does not take into account the molecular interactions between polymer and drug molecules [77]. PM show a melting transition at 166.72 ºC higher than the Tg of HPMCAS LF, indicating the crystalline nature of ITZ within the mixture. Also for ITZ:HPMCAS LF, there is a complete miscibility between drug and polymer. Additionally, no melting peak is present in the thermogram, which validates the amorphous form of the SDD.

Similarly, the thermogram of SDD ITZ:PVP/VA (tjhermogram I in Figure 3.9 B- Right) presents a single Tg at 91.03 ºC which implies the presence of a single phase. As in the previous SDD, the obtained Tg is an intermediate value between the ITZ and polymer Tgs, together with the absence of a melting peak in the thermogram, proves complete miscibility between the drug and the polymer. The estimated Tg using the Gordon-Taylor/Kelley-Bueche GTKB equation 90.22 ºC, which is in agreement with the experimental one. The respective physical mixture shows a melting endotherm at 165.49 ºC after Tg of PVP/VA (125.19 ºC), indicating the crystalline nature of ITZ within the mixture.

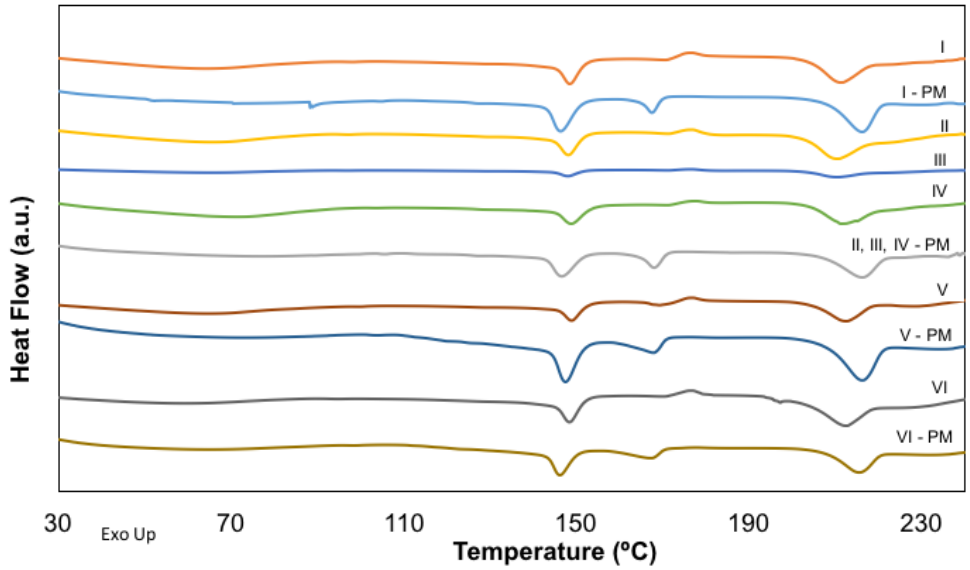


Figure 3.10 – DSC thermograms of ITZ tablet formulations. I: Formulation 1; I - PM: Physical mixture of Formulation 1; II: Formulation 2 – 212 μm; III: Formulation 2 – 600 μm; IV: Formulation 2 – 800 μm; II, III, IV - PM: Physical mixture of Formulation 2; V: Formulation 3; V – PM: Physical mixture of Formulation 3; VI: Formulation 4; VI – PM: Physical mixture of Formulation 4.

Melting endotherms, corresponding to crystalline excipients, in ITZ tablet formulation thermograms are present in physical mixtures thermograms with the absence of ITZ melting (Figure 3.10). This excludes the possibility of polymorph formation due to tableting operation (as suggested by XRPD analysis) and corroborates the hypothesis that ITZ is present in the amorphous form. The presence of an exothermic peak at around 175°C, should correspond to the transformation of amorphous ITZ into its crystalline form, since this exotherm is not present in physical mixtures thermograms.

Particle Morphology and Size

Concerning the assessment of particle morphology and size of ITZ, polymers PVP/VA and HPMCAS LF and generated SDDs, the respective micrographs are shown in Figure 3.11.

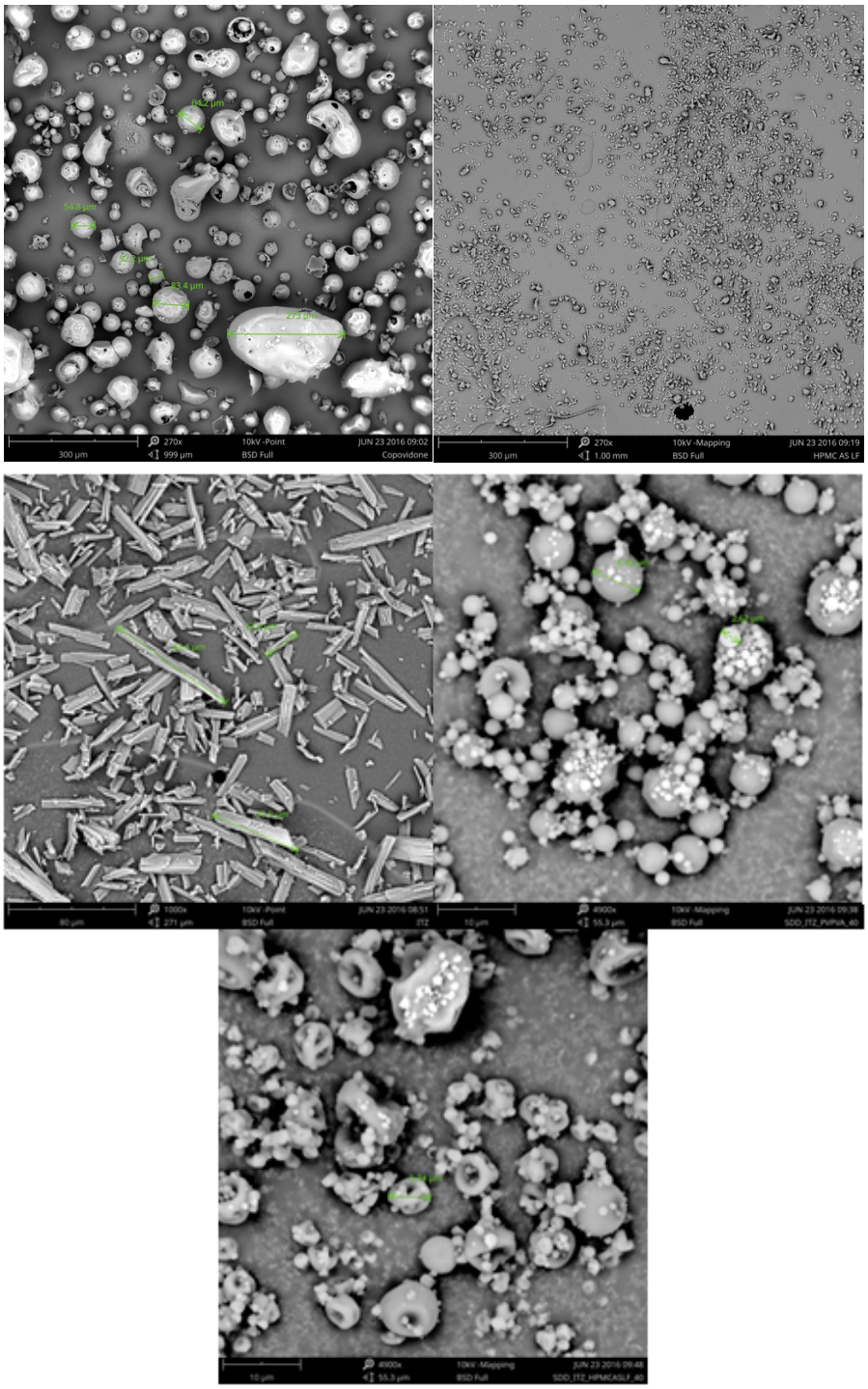


Figure 3.11 – SEM Micrographs of raw PVP/VA, HPMCAS LF, ITZ, ITZ:PVP/VA SDD at a 40% drug load and ITZ:HPMCAS LF SDD at a 40% drug load at different magnifications.

The particles of crystalline ITZ were found to be large orthorhombic crystals with a particle size ranging from 10 µm to 70 µm. PVP/VA particles are spherical and larger than HPMCAS LF, that have an irregular shape. Compared to the starting material, the particle sizes of the SDDs are much smaller. SDD ITZ:PVP/VA solid dispersions prepared by spray drying were spherical in shape. The particulate structure of the SDD ITZ:HPMCAS LF had a partially collapsed structure that can be described as deflated spherical particles (buckled particles). The particle morphology results from the balance between the evaporation rate of the solvent and the diffusion rate of the solutes, quantified by the Peclet number, evaporation rate/diffusion rate ratio. If the drying is diffusion controlled (low Peclet number) buckling can occur [78]. Thus, the different solvents used in both SDDs would explain the different particle morphologies observed, i.e. the DCM, the solvent utilized with PVP/VA polymer, possesses a lower vaporization heat, promoting a faster drying step – evaporation rate controlled – high Peclet number, which results in spherical particles. On opposite, in the case of the SDD ITZ:HPMCAS LF, the solvent employed, MeOH, has a higher vaporization rate, resulting in a slower droplet drying – diffusion controlled drying, which justifies the observed buckled particles. Another hypothesis, is that the different morphology of the two SDDs produced is related to the different mechanical properties of the polymers, PVP/VA and HPMCAS LF. Both SDDs show small particles (nano range) on the surface of the larger ones which suggests a large particle size span, that will impact the dissolution rate of these powders.

The particle size of both SDD formulations were obtained by laser diffraction and the corresponding distribution curves can be seen in Figures 3.12 and 3.13.

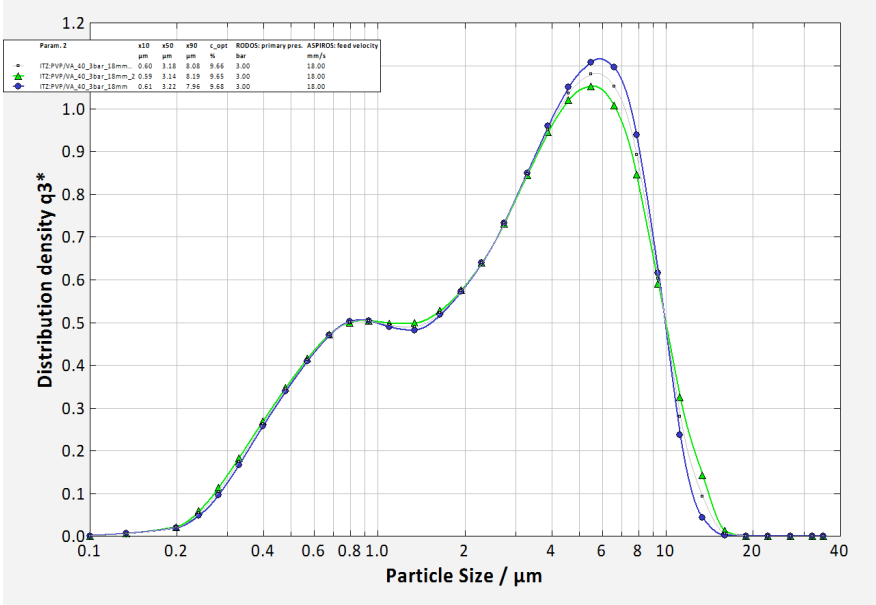


Figure 3.12 – Particle Size Distribution of the SDD ITZ:PVP/VA at 40% of drug load, obtained by laser diffraction.

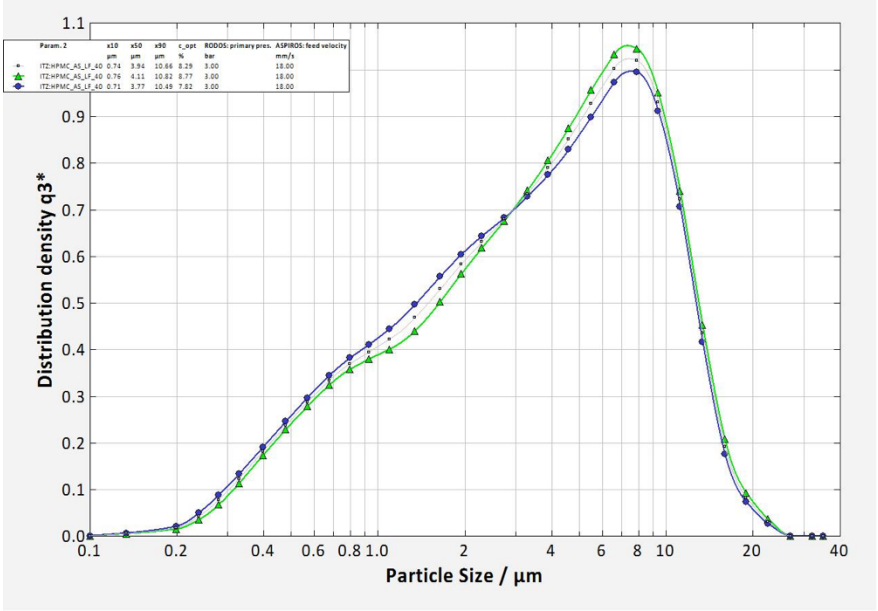


Figure 3.13 – Particle Size Distribution of the SDD ITZ:HPMCAS LF at 40% of drug load obtained by laser diffraction.

Both SDDs show a considerable span, relative span factors (RSFs) of 2.3 and 2.5 for SDD ITZ:PVP/VA and ITZ:HPMCAS LF, respectively. For a two fluid atomizer, the RSFs obtained are acceptable [79]. The first one shows a maximum of distribution density at 7 μm and a D50 of 3.94 μm while the second one has two ends of the function at 0.8 and 6 μm and a D50 of 3.18 μm. The particle size of the particles is smaller than it would be expectable based on the atomization model developed by Hovione. Although this can influence the compression and dissolution process, as the two powders had similar particle sizes, this fact has no significant effect in the current work.

Particle size analysis by analytical sieving of formulations (Figure 3.14) was conducted in order to assess granule distribution. Figure 3.14 shows a wide span of particle size due to the granulation method, i.e., manually milling the tablets through sieves of a fixed MESH. The high fine percentage present in the formulations may cause a burst, i.e. quick dissolution in the beginning of the test. The variability present in dissolution profiles, presented in the next section, may be due to the large span of particle size distribution, since they have different surface areas, i.e. dissolution rates [16]. Nevertheless, formulations granulated using the same sieve (Figure 3.14 – Left) exhibit an identical PSD whereas the formulation divided and granulated using different sieves present considerable three distinct distributions. Therefore, the goal of obtaining six different formulations was achieved: four formulations with different compositions have identical PSDs (Figure 3.14 – Left) and three formulations with the same composition, but different PSDs were obtained (Figure 3.14 – Right), which will allow to assess the excipients/particle size effect on dissolution.

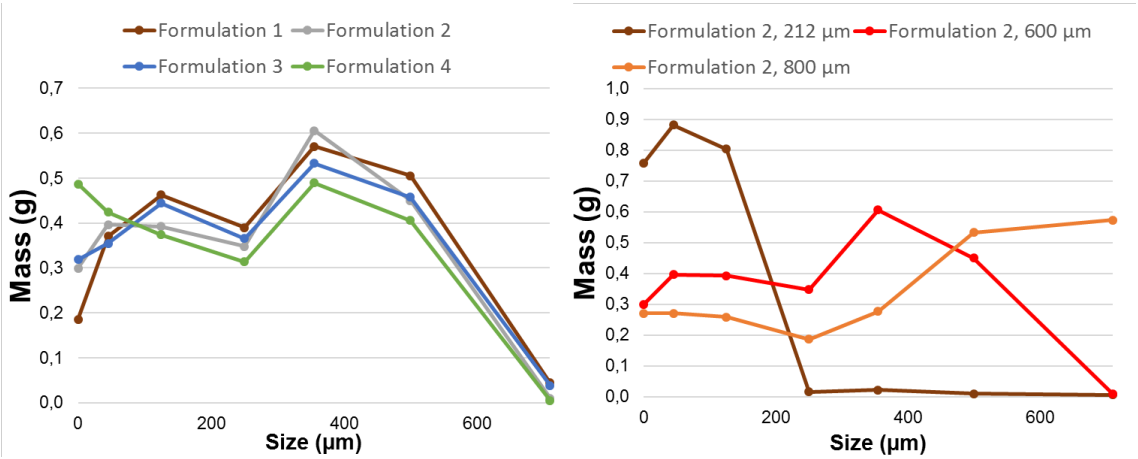


Figure 3.14 – Particle Size Distribution of the six formulations of ITZ tablets, obtained by analytical sieving. Left: Formulations with identical particle size distribution - Formulation 1 (brown), formulation 2 (grey), formulation 3 (blue) and formulation 4 (green). Right: Formulations with distinct particle size distribution - Formulation 2 - 212 µm, formulation 2 - 600 µm (red), formulation 2 - 800 µm (orange),

3.5. SDD Dissolution

In order to assess the effect of sodium taurocholate and lecithin, components of biorelevant media, in ITZ dissolution, media without these compounds were also tested and are denoted as Blank. This was only applied to intestinal media, since the gastric medium (FaSSGF) has low amounts of these compounds when compared to the intestinal media (as mentioned previously in Chapter 1)

The dissolution profile of the drug product intermediates was assessed using biorelevant media with a pH value in accordance to the preferential solubility of the polymer used: gastric media representing the fasted stomach, FaSSGF for ITZ:PVP/VA SDD formulation and the fasted intestine, FaSSIF for ITZ:HPMCAS LF SDD formulation (enteric formulation). Figure 3.15 shows dissolution profiles for both SDD formulations in these biorelevant media.

Due to the low solubility of ITZ in the tested media, it was not possible to achieve sink conditions in the dissolution experiments with the 900 mL of media. From the dissolution profile presented, it can be seen that in ITZ:PVP/VA SDD formulation, ITZ quickly dissolves, but starts precipitating after 1h. This illustrates quite well the “spring and parachute” effect. Regarding the enteric ITZ:HPMCAS LF SDD formulation, a higher solubility was obtained in FaSSIF (pH 6.5) even though, being a weak base. ITZ possesses a higher solubility in the pH of FaSSGF (pH 1.6), rather than in the intestinal media. This behavior might be attributed to the HPMCAS LF ability to stabilize the ITZ in solution, in contrast to PVP/VA, which was not a good supersaturation stabilizer (“parachute”) since ITZ started to precipitate after one hour of the test.

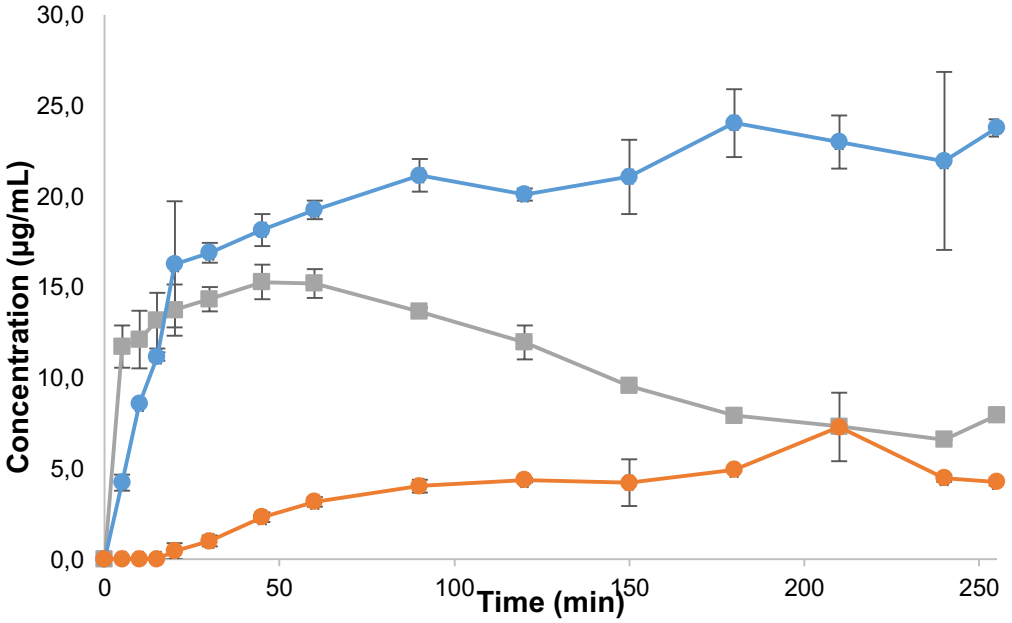


Figure 3.15 – Dissolution profiles of SDD ITZ:PVP/VA in FaSSGF (grey), SDD ITZ:HPMCAS LF in FaSSIF (blue) and SDD ITZ:HPMCAS LF in Blank FaSSIF (orange). The vertical bars correspond to the standard deviation from duplicates (n=2).

The difference observed between FaSSIF and Blank FaSSIF media (4-fold ITZ solubility increase) is explained by the presence of lecithin and sodium taurocholate in FaSSIF, which solubilize ITZ into mixed micelles [80]. The bile components exert their effect on solubility by forming different colloidal phases that are able to solubilize the ITZ molecule better, therefore, the mixed micelles present in FaSSIF produced a higher solubility than in the correspondent buffer (blank FaSSIF) .

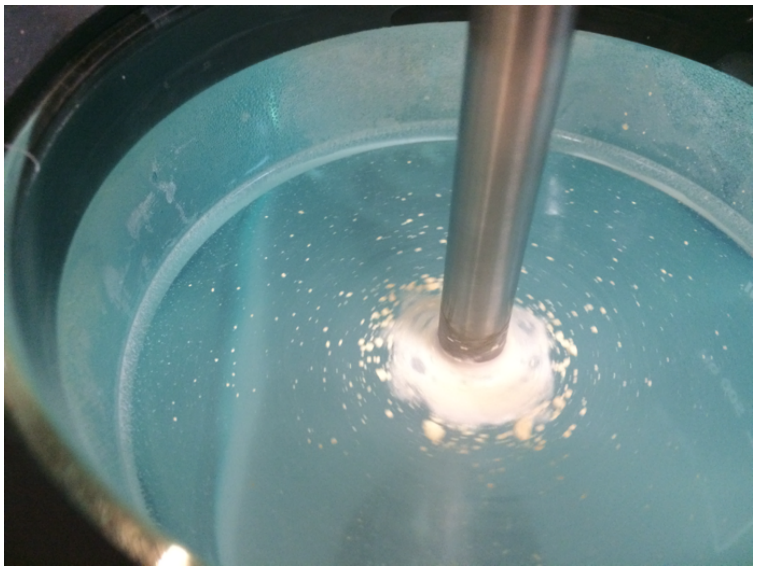


Figure 3.16 – Floating powder during ITZ SDD dissolution testing.

It is important to highlight that the SDD powders floated during the performed tests (illustrated in Figure 3.16), which is quite common when evaluating the dissolution profiles of powders

using USP Apparatus 2. This behavior is even more noticeable in SDD powders due to their traditional lack of wettability. Some alternatives may be employed to overcome this issue, for example the preparation of suspensions that are then added to the dissolution media, the inverse order of addition of sample/dissolution media and, as mentioned before in the Chapter 1, the use of USP Apparatus 4.

3.6. Granule Dissolution

The dissolution profiles of the drug products - ITZ tablets - were assessed using biorelevant media, by means of USP Apparatus 2, under non sink conditions (100 mg of ITZ to 900 mL of dissolution medium). Accordingly, the linear portion of each curve (Figures 3.17, 3.19, 3.20, and 3.21) is quite brief as saturation solubility is quickly reached. Although sink conditions are desirable throughout dissolution process, they may not prevail in GI tract, as they depend on the composition and volume of the fluids of the GI tract and on the permeability of the drug [72].

3.6.1. Granule size effect in dissolution profile

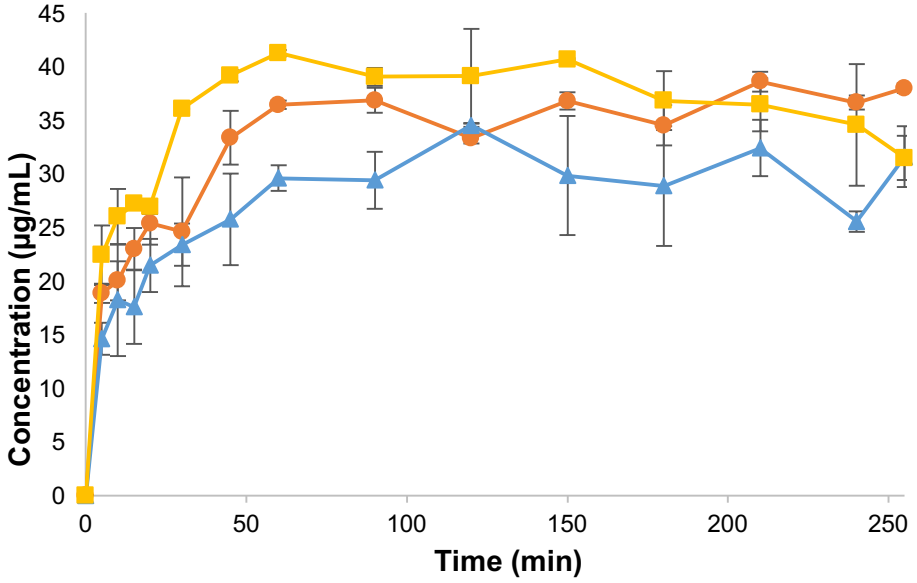


Figure 3.17 – Dissolution profiles of formulation 2 - 212 µm (yellow), formulation 2 - 600 µm (orange) and formulation 2 - 800 µm (blue) in FaSSGF. The vertical bars correspond to the standard deviation from duplicates (n=2).

Figure 3.17 represents the influence of particle size in dissolution of the non-enteric formulation (containing the SDD ITZ:PVP/VA), with HPMC as binder (formulation 2) - in gastric media. It is observable that the formulation 2 with smallest particle size - 212 µm, exhibited the most rapid dissolution rate with approximately 68% of ITZ in solution at 30 min, followed by the formulation 2 - 600 µm. This was expectable since, according to the Noyes Whitney model, the dissolution rate is inversely proportional to the particle size due to the increase on surface area [16].

Even though these tests were carried out under non sink conditions, and thus the saturation solubility was quickly achieved, it was still possible to discriminate the particle size effect on the dissolution profile.

Figures 3.17 and 3.19 show that ITZ dissolves quickly in gastric media, since it is a weak base, ionized, and therefore more soluble, at low pHs.

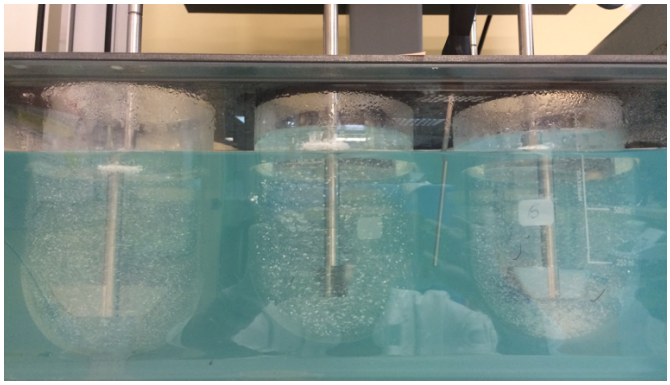


Figure 3.18 – Granules dissolution testing.

3.6.2. Dissolution profile of non enteric formulations – binder effect

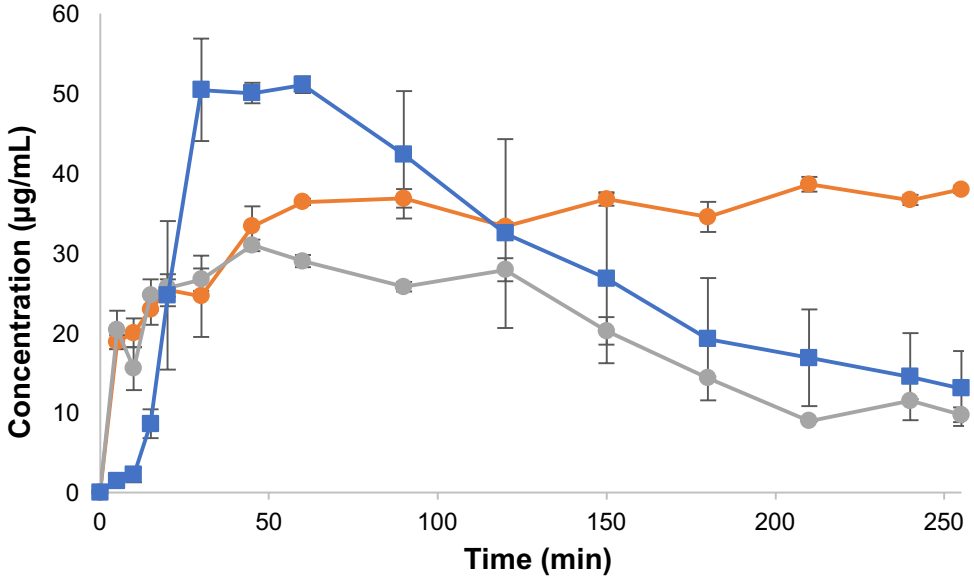


Figure 3.19 – Dissolution profiles of formulation 2 - 600 µm (orange), formulation 1 (grey) and SPO (blue) in FaSSGF. The vertical bars correspond to the standard deviation from duplicates (n=2).

Still regarding the fasted gastric environment, and therefore, the non-enteric formulations (Figure 3.19), it is seen that SPO dissolves until saturation solubility at a lower dissolution rate than formulations 1 and 2 of the same particle size, which might be due to the dissolution of the spheres sugar coating, delaying ITZ dissolution and maintaining the supersaturation only for 30 min. Although not reaching the same saturation level of SPO, formulations 1 and 2 seem to have

greater capacity of continually releasing ITZ. Formulation 1 releases only a half of the ITZ comparing to SPO (approximately 31 µg/mL instead of approximately 52 µg/mL), but takes about 75 min for a decrease in ITZ concentration starts to be detected. Formulation 2 holds the saturation solubility during the four hours of the test. Since the only different aspect among these last two formulations is the binder (MCC for formulation 1 and HPMC for formulation 2), it is reasonable to infer that the precipitation inhibiting capacity of HMPC prevents the precipitation of ITZ to occur.

3.6.3. Dissolution profile of the enteric formulations - binder and solubilizers effect

The enteric formulations (formulation 3 and 4) dissolution profiles were determined in FaSSIF (fasted intestinal medium) and blank FaSSIF (the latter corresponding to the buffer of FaSSIF alone). The difference between these two formulations is the binder used: MCC for formulation 3 and HPMC for formulation 4.

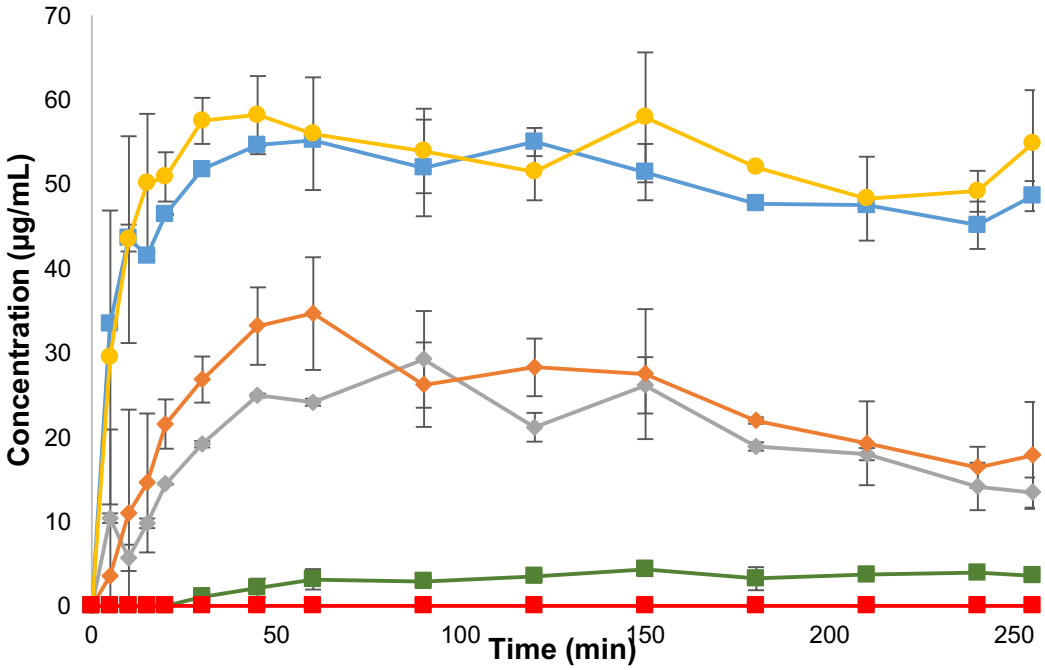


Figure 3.20 – Dissolution profiles of formulation 4 (yellow), formulation 3 (blue) and SPO (green) in FaSSIF, formulation 4 (orange), formulation 3 (grey) and SPO (red) in Blank FaSSIF. The vertical bars correspond to the standard deviation from duplicates (n=2).

Comparing the dissolution profiles of formulation 3 and 4 in FaSSIF, the effect of the media solubilizers is a 2-fold increase in saturation solubility (at the same time point, 90 min, for formulation 3 a difference of concentration from 29.20 µg/mL to 51.89 µg/mL; for formulation 4 a difference of concentration from 26.20 µg/mL to 53.88 µg/mL). As already mentioned, this difference is due to the presence of the biological solubilizers, lecithin and sodium taurocholate, in FaSSIF, which solubilize ITZ into mixed micelles [80]. In fact, these compounds are known to have a significant enhancing effect upon the dissolution rate of poorly soluble drugs by improving the

wettability of solids and by increasing the solubility of a drug substance into mixed micelles [80]. This micellar solubilization of ITZ in FaSSIF provided the high “spring effect” and the HPMCAS LF acts as a precipitation inhibitor to sustain the “parachute effect”, preventing the nucleation and fast precipitation of ITZ in FaSSIF. The same effect is observed when comparing dissolution profile of SPO in Blank FaSSIF versus FaSSIF. The results, shown in Figure 3.15 and once again confirmed by the ones presented in Figure 3.20, show that the presence of lecithin and sodium taurocholate clearly produced an enhancement in dissolution compared with the compendial media. Thus, the dissolution rate of poorly soluble, lipophilic drugs such as ITZ may be improved greatly in biorelevant media in comparison to the dissolution rate observed in simple aqueous solutions. In Figure 3.20, representing the intestinal media, it is possible to observe that enteric formulations 3 e 4 have similar releases: even though they have different binders. The similarity between these profiles, may be due to the polymer, HPMCAS LF, which is well known for its capacity of stabilizing the amorphous ITZ in solution [81], overlying the binder effect.

3.6.4. Food effects assessment

The bile components exert their effect on solubility by forming different colloidal phases that could solubilize lipophilic drugs. *In vivo*, food stimulates the release of bile salts and phospholipids; this highlights the importance of the fed state in solubilizing this lipophilic compound. Thus, food effects were also assessed regarding the intestinal environment: the dissolution profile of formulation 4 (enteric polymer with HPMC binder) was determined in FaSSIF and FeSSIF (Figure 4.26). Formulation 4 was selected for this experiment since, although smooth, the saturation solubility of ITZ is slightly higher, when compared to formulation 3 (57 μg/mL vs. 52 μg/ml, respectively).

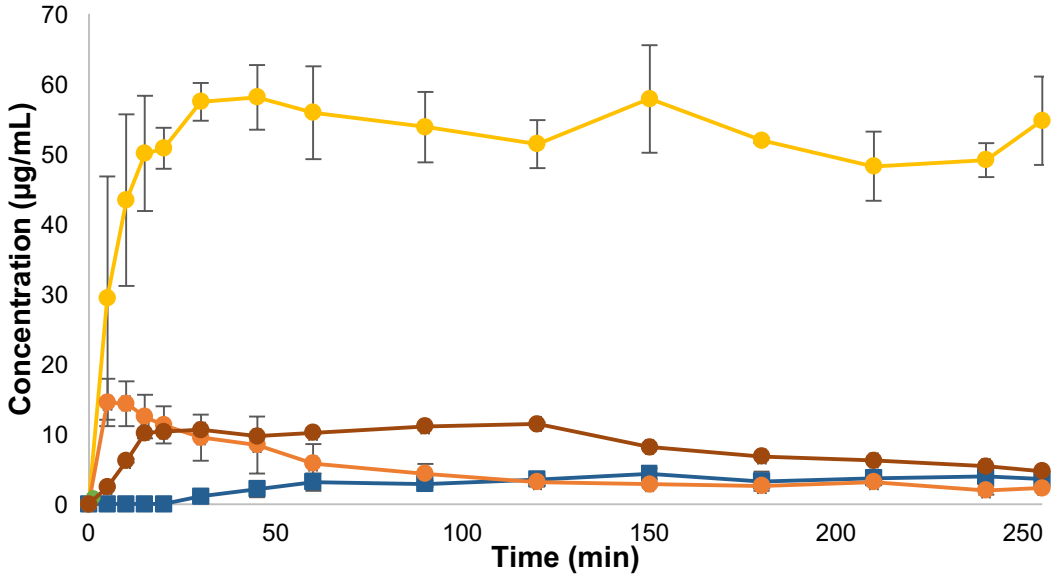


Figure 3.21 – Dissolution profiles of formulation 4 (yellow) and SPO (blue) in FaSSIF, formulation 4 (orange) and SPO (brown) in FeSSIF. The vertical bars correspond to the standard deviation from duplicates (n=2).

The SPO concentration in FeSSIF is more than 3 times higher than in FaSSIF (at 10 min, 10.35 μg/ml in FeSSIF against no detection in FaSSIF) and ITZ concentration starts decreasing after 2 hours from the start of the test (Figure 3.21). This was expectable because of the higher concentration of solubilizers, lecithin and sodium taurocholate. Formulation 4 shows the opposite behavior: at 30 minutes, the ITZ concentration in FaSSIF is approximately 57 μg/ml, whereas in FeSSIF is of about 9 μg/ml, at the same time point.

It is important to note that, FaSSIF and FeSSIF are adjusted to different pH values: whilst FaSSIF is used at pH 6.5, FeSSIF is prepared at pH 5.8. The different ITZ solubility in these two media might be due to the fact that this formulation is enteric: HPMCAS LF starts dissolving at 5.5, so its solubility must be lower in 5.8 than in 6.5. Therefore, the effect observed in formulation 4 may be to the fact that ITZ release from the polymeric matrix is being limited by the polymer solubility in the medium.

In SPO patient medical information, it is recommended to take the dosage form after full meals [82]. Also, it is said that ITZ's bioavailability is lowered in individuals with reduced gastric acidity. This is in apparent contrast, since after meals the gastric pH is increased. The reason pointed to the increased BA in the fed state is the presence of solubilizers present in the food and bile salts and lecithin. Indeed, ITZ solubility in FeSSIF was higher than in FaSSIF. Although it is also necessary to compare dissolution profiles of ITZ in the fed and fasted stomach and to quantify the free ITZ. Soon biorelevant.com will launch FeSSGF, a standardized medium representing the fed stomach, thus, to investigate this question, it would be interesting to perform a pH shift experiment simulating the fed state and to use ultracentrifugation to distinguish molecularly dissolved ITZ.

3.6.5. pH effect in dissolution profile

To assess the impact of pH in the dissolution profile of the reference drug product, SPO, its dissolution was performed in four different gastric media: SGFsp - pH 1.2, FaSSGF - pH 1.2 and 1.6, SGFsp - pH 1.6 (Figure 4.22).

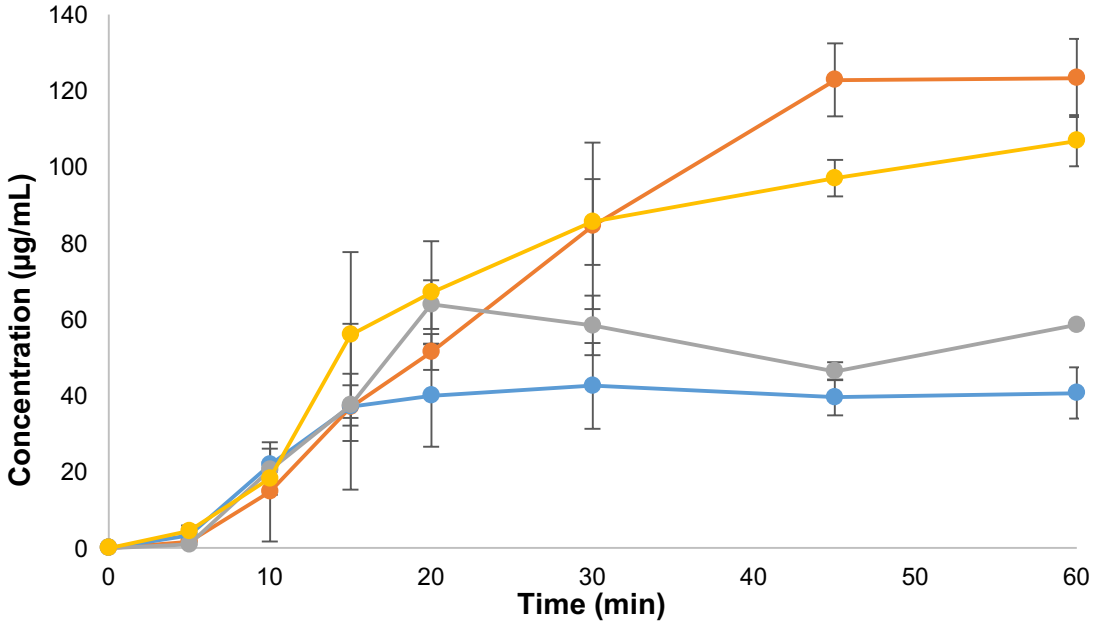


Figure 3.22 – Dissolution profiles of SPO in SGFsp – pH 1.2 (orange), FaSSGF - pH 1.2 (yellow), SGFsp - pH 1.6 (grey) and FaSSGF – pH 1.6 (blue). The vertical bars correspond to the standard deviation from duplicates (n=2).

In the FaSSGF medium, high release of ITZ from SPO was observed, reaching around 40 µg/mL. However, this corresponded to around 30% of SPO ITZ release in SGFsp (123 µg/mL) after one hour. Since FaSSGF is also an acidic medium, additionally composed by lecithin and sodium taurocholate, this fact was strange and could only be attributed to the pH difference (1.6 instead of 1.2) between these media. In order to explain this result, an additional experiment was conducted: dissolution of SPO in HCl 1.6 and in FaSSGF which pH was adjusted to 1.6. Indeed, when the pH increased, ITZ is less ionized (pKa value of the conjugate acid form of ITZ is 3.7) and therefore less soluble [83]. This test proved that a slight pH variation can impact severely ITZ dissolution (Figure 3.27) and that the pH effect is clearly dominant when compared to the solubilizers effect, since although its presence in FaSSGF and absence in SGFsp, higher releases were observed in the last. It is worth noting that the sodium taurocholate and lecithin concentrations in FaSSGF (medium simulating the fasted stomach) are residual when comparing to the media simulating the intestine, i.e. they are 38 and 188 times higher in the fasted and fed intestine, respectively.

3.6.6. Biorelevant pH shift of the best formulations

In order to assess the dissolution profile taking into consideration the different pHs that an oral drug will encounter, a pH shift method was employed. In this method, SPO, Formulation 4 (enteric polymer, HPMC binder) and Formulation 2 (non enteric polymer, HPMC binder), were used (Fig 4.23). These two were chosen because of their superior performance, i.e., higher solubility maintained for a higher period of time (Figures 3.19 and 3.20).

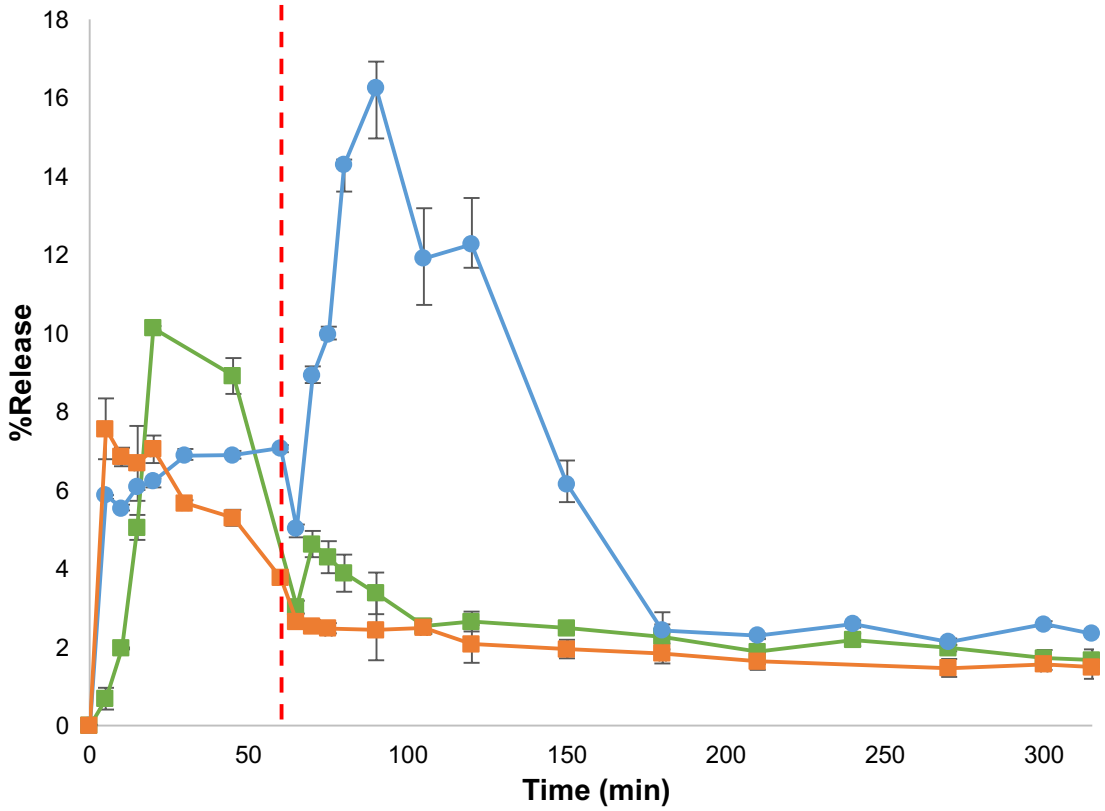


Figure 3.23 – pH shift of SPO (green), formulation 2 – 600 μm (orange) and formulation 4 (blue) from FaSSGF to FaSSIF. The dashed line corresponds to the moment of pH shift. The vertical bars correspond to the standard deviation from duplicates (n=2).

As expected, non pH-dependent formulation 2, releases ITZ at low pH (gastric), however, upon pH change, an extensive precipitation of the drug occurs. Near complete precipitation occurred for all formulations 1h after pH transition. Due to a very low pKa value of the conjugate acid form of ITZ, the change in pH from the acidic environment of pH 1.6 to a higher pH of 6.5 in the dissolution medium resulted in the conversion of ITZ to its neutral form. As the intrinsic solubility of non-protonated drugs is extremely low, it has precipitated out from the dissolution medium at pH 6.5. These results suggest that pH is a more dominant factor in influencing ITZ precipitation when compared to the micellar solubilization with the bile salts and lecithin as it was stated in the analysis of Figure 3.22.

In contrast, after pH shift, the enteric formulation 4, shows an increases of ITZ in solution at about 16 ± 3 % at 90 minutes, followed by a precipitation pattern from 90 to 180 minutes, where it reaches the same concentration as SPO. Formulation 4 has the best performance of all the formulations tested, since it achieves the maximum of release in the main site of absorption for ITZ, i.e. duodenum [84]. These pH shift tests involved an immediate change from gastric pH to intestinal pH, however, the rate at which the contents of the stomach emptied into the intestine should also be considered [46]. In this way, the pH shift approach, employing USP Apparatus 2 (or "dumping method"), should be considered as the worst case, when testing weakly basic drugs due to accelerated precipitation kinetics and the opposite when testing acidic drugs. To better assess the *in vivo* precipitation behavior of ITZ, both transfer model and USP Apparatus 4 should be employed. USP Apparatus 4 has the additional possibility of maintaining sink conditions throughout the test.

These *in vitro* dissolution profiles in biorelevant media containing surfactants quantify all the drug species that are formed in solution, including drug-polymer colloids and micelles that may not be able to permeate through the intestinal barrier [85]. Consequently, it is of extreme importance to quantify the concentration of molecularly ITZ dissolved (true supersaturation), since this is the key parameter affecting the first step of bioavailability: absorption.

3.7. Free drug quantification tests

Before presenting the results regarding the dialysis membrane experiments, the workflow to obtain the drug apparent permeability coefficient and the free drug concentration using *in vitro* dissolution data and dialysis membranes will be described in this section.

Considering two compartments (1 and 2, denoted as donor and acceptor, respectively) with different volumes (V_1 and V_2) and concentrations (C_1 and C_2) separated by a porous membrane of area A (Figure 3.24), the mass flux through the membrane is given by:

$$J = \frac{D}{\delta}(C_1 - C_2) \quad \textbf{(2)}$$

J: API mass flux through the membrane ($kg.m^{-2}.s^{-1}$);
D: diffusion coefficient of the API in the membrane ($m^2.s^{-1}$);
δ: membrane thickness (m);
C_1: free API concentration in the donor compartment ($kg.m^{-3}$);
C_2: free API concentration in the acceptor compartment ($kg.m^{-3}$).

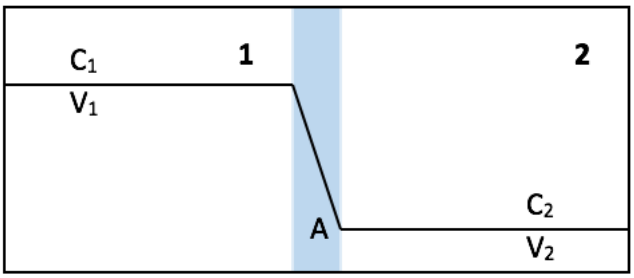


Figure 3.24 – Representation of the mass transport across a membrane model.

The free API within the donor compartment (1) is given by the mass balance between the free API present in its reservoirs (tablet granules, micelles and colloids) and permeated free compartment (2). Accordingly, the concentration in the acceptor compartment is given by the free API mass flux across the membrane:

$$\begin{cases} V_1 \dfrac{dC_1(t)}{dt} = -V_2 \dfrac{dC_2(t)}{dt} + f(t) & \textbf{(3)} \\ V_2 \dfrac{dC_2(t)}{dt} = JA = \dfrac{DA}{\delta}(C_1 - C_2) & \textbf{(4)} \end{cases}$$

f(t): term corresponding to the free drug release from its reservoirs (tablet granules/micelles/colloids);
A: membrane surface area;
V_1: volume of the donor compartment (m^3);
V_2: volume of the acceptor compartment (m^3);
t: dissolution time (s).

It is not possible to solve the first equation for the free API concentration in the donor compartment (C_1), since the term corresponding to the rate of release of free API present in its reservoirs (tablet granules, micelles and colloids) is not known *a priori*. Nevertheless, the free API concentration in the donor compartment (C_2) is measured in the dialysis membranes experiments, therefore it is possible to solve the Equation 4 for the free API concentration in the donor compartment (C_1) whose integral will correspond to the total amount of API potentially capable of being absorbed.

$$V_2 \frac{dC_2(t)}{dt} = \frac{DA}{\delta}(C_1 - C_2) \quad \textbf{(5)}$$

$$\Leftrightarrow \frac{V_2 \delta}{DA}\frac{dC_2(t)}{dt} = (C_1 - C_2) \quad \textbf{(6)}$$

$$\Leftrightarrow C_1 = C_2 + \frac{V_2 \delta}{DA}\frac{dC_2(t)}{dt} \quad \textbf{(7)}$$

$$\Leftrightarrow C_1 = C_2 + \frac{1}{K}\frac{dC_2(t)}{dt} \quad \textbf{(8)}$$

$$\Leftrightarrow \int_0^t C_1(t)\,dt = \int_0^t C_2(t)\,dt + \frac{1}{K}(C_2(t) + C_0) \quad \textbf{(9)}$$

since when $t = 0$, $C_2 = 0 \Longrightarrow\ C_0 = 0$.

$$\Leftrightarrow \int_0^t C_1(t)\,dt = \int_0^t C_2(t)\,dt + \frac{C_2(t)}{K} \quad \textbf{(10)}$$

$\int_0^t C_1(t)\,dt = total\ amount\ of\ free\ drug\ released\ from\ the\ dosage\ form$

K: API apparent permeability (s^{-1}).

The first step (Figure 3.25) is to determine the API apparent permeability – K (Equation 8). This is achieved by performing an experiment in USP apparatus 2, controlled temperature and stirring speed, using a crystalline API (known free drug fraction) saturated solution (C_1 is the API solubility in the medium tested) and one membrane per time point (to generate C_2 cumulative results), using at least three time points. It is important to ensure that the dialysis membranes have similar dimensions, since this constant is dependent of the exposed membrane area. Additionally, it is essential to confirm that the permeability rate is similar to the dissolution rate (i.e. permeation is not the rate limiting step), so that when testing SDDs containing formulations, the API is able to permeate the membrane before its precipitation (recall that amorphous APIs have metastable solubilities).

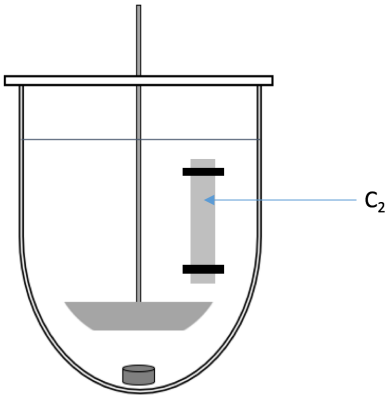


Figure 3.25 - Representation of a dissolution and permeation test using the reverse dialysis method. C_2 represents the drug concentration in the permeate.

The next step is to perform the dissolution test with dialysis membranes, in the same conditions as the ones applied in the previous dissolution test for the dosage form under study and obtain the API permeate concentration (C_2). Afterwards, the free drug concentration (C_1) can be estimated using Equation 10.

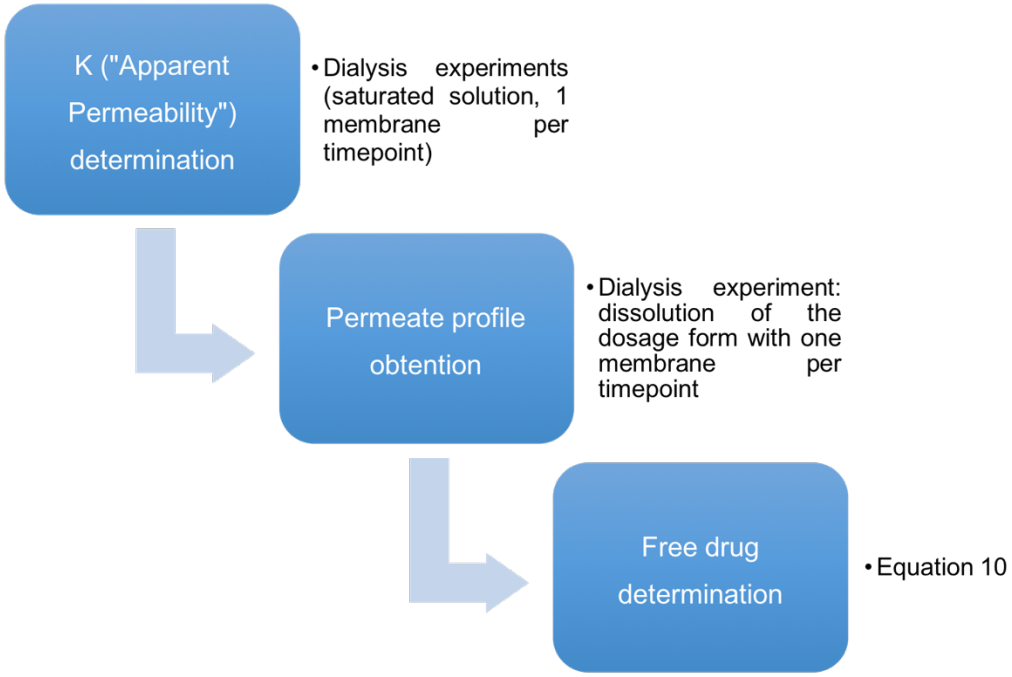


Figure 3.26 – Free drug assessment workflow.

3.7.1. API permeation tests

An initial step was the evaluation of the crystalline ITZ ability to permeate the dialysis membrane (Figure 4.29).

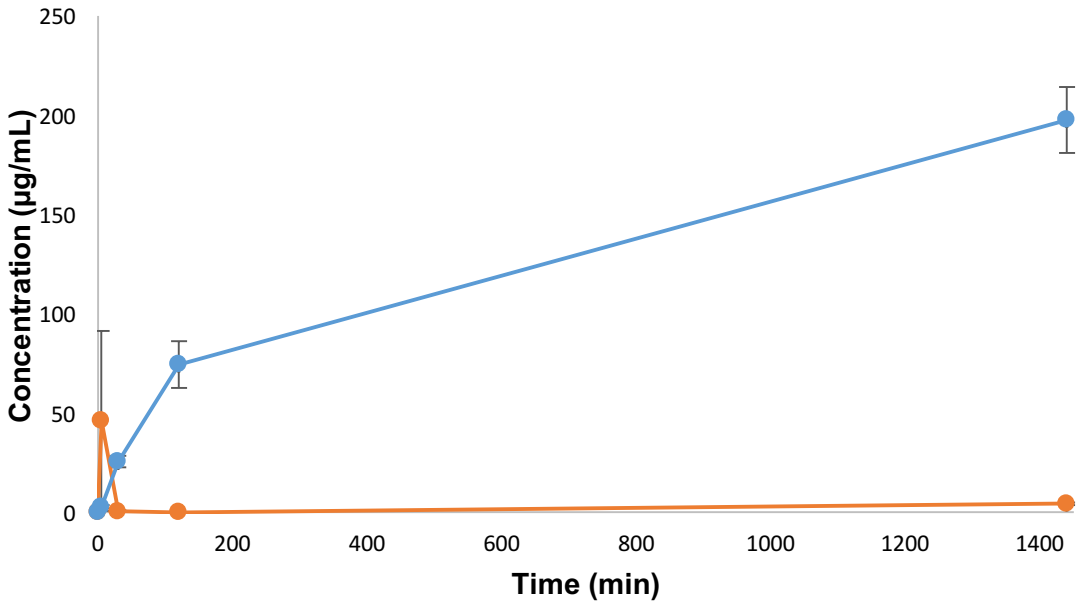


Figure 3.27 – Dissolution (blue) and permeation (orange) profiles of crystalline ITZ in HCl 1.6 with 1% of SDS. The vertical bars correspond to the standard deviation from duplicates (n=2).

No significant permeation of crystalline ITZ was detected after 24 hours (Figure 3.27). It was hypothesized the main fraction of the ITZ was being solubilized by micelles of SDS and

consequently could not pass through the dialysis membranes. To assess this, the test was repeated with SPO in SGFsp (medium without surfactants) and the same result was obtained, eliminating the micelles hypothesis. The high concentration value inside the membrane obtained at 5 minutes (46 μg/mL) is probably due to sample contamination. These tests with dialysis membranes (Figures 3.27 and 3.28) were carried out with very acidic media, 1.2 and 1.6. The membranes manufacturer recommended pH range between 2 and 9. It may be possible that this low pHs could have damaged the membranes, even though their visual appearance remained identical.

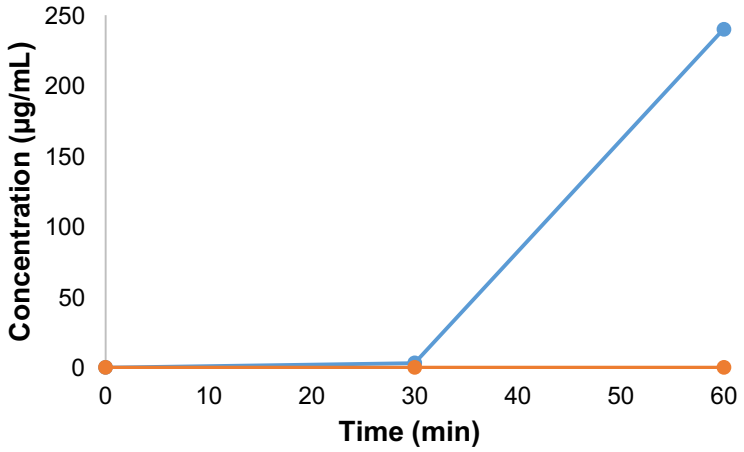


Figure 3.28 – Dissolution (blue) and permeation (orange) profiles of crystalline ITZ in SGFsp.

3.7.2. SDD and tablet permeation tests

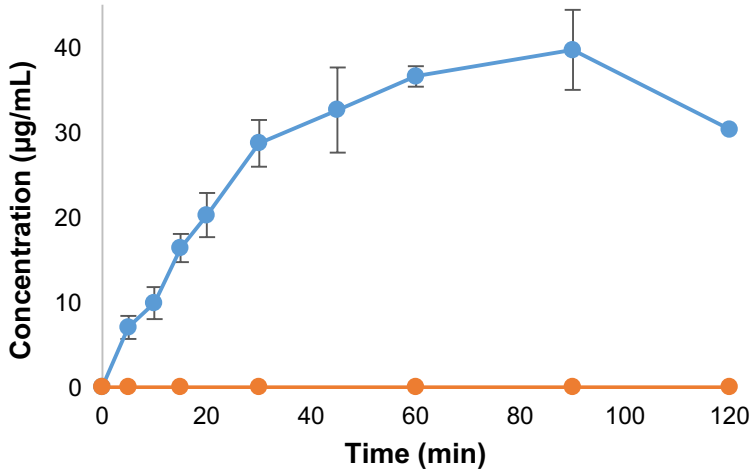


Figure 3.29 – Dissolution (blue) and permeation (orange) of SDD ITZ:PVP/VA in FaSSGF. The vertical bars correspond to the standard deviation from duplicates (n=2).

Dissolution profiles of the SDDs (Figures 3.29 and 3.30) display higher concentrations than the ones obtained in previous dissolution tests (section 3.5). This is probably due to the membranes movement within the dissolution vessel, which improved the powder wettability. These profiles confirm the hypothesis that the saturation solubility was not achieved on the previous

SDDs dissolution experiments also supported by tablet dissolution experiments, where higher concentrations were obtained in the same media. Still, some aggregates of floating powder were observed (Figure 3.31). Moreover, any free drug was quantified and the experiment with the intestinal media (pH 6.5) discarded the hypothesis of the pH damaging the membranes.

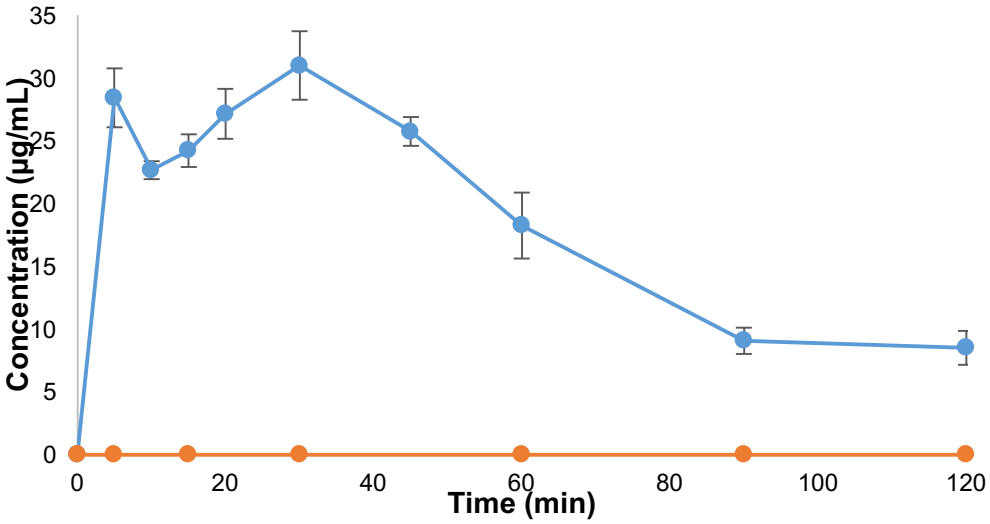


Figure 3.30 – Dissolution (blue) and permeation (orange) of SDD ITZ:HPMCAS LF in FaSSIF. The vertical bars correspond to the standard deviation from duplicates (n=2).

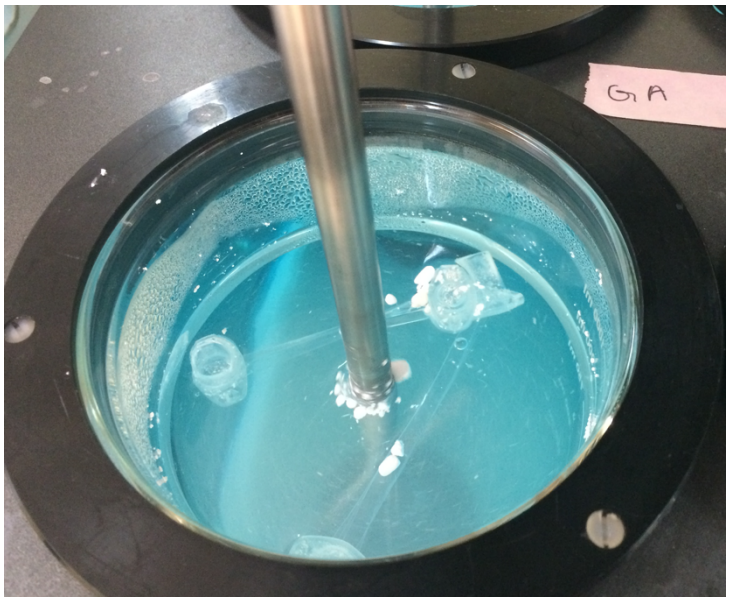


Figure 3.31 – SDD dissolution and permeation experiment.

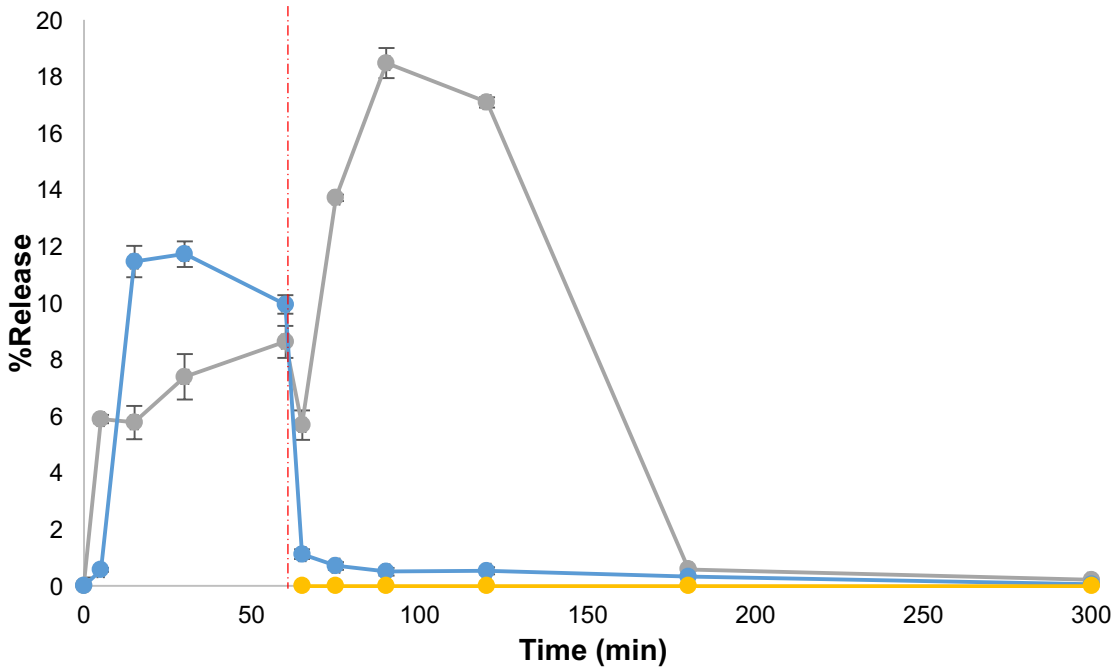


Figure 3.32 – Dissolution profile of a pH shift experiment of SPO (blue) and formulation 4 (grey) and permeation profile of these formulations (yellow). The dashed line corresponds to the moment of pH shift. The vertical bars correspond to the standard deviation from duplicates (n=2).

The pH shift experiment showed similar results (Figure 3.32) to the ones obtained in the previous pH shift (section 3.6.6.) and it was not possible to quantify free ITZ.

The goal of these experiments, free API quantification, was not achieved. In order to investigate the reason for this, there are some aspects that can be tested in the future. It has been suggested that the molecular weight cutoff (MWCO) of the dialysis membranes should be about a hundred times the size of the drug molecule [86]. Therefore, a membrane with a MWCO of at least 80 kD should be tested. The MWCO of the dialysis membranes seems suitable to isolate the free ITZ from the polymer and micelles. Two hypothesis are proposed: the first is the possibility of interaction between the API and membrane resulting in its retention. The second one, is that the API permeation depends on the method applied: conventional or reverse dialysis, due to the membrane properties (e.g. manufacturing material and pore geometry). This can be assessed by repeating the API test with it placed inside the membrane. Before future experiments, the potential for the drug to bind to the dialysis membrane should be assessed.

This method demonstrated severe limitations: possibility of the permeate concentration being below the LOQ of the quantification method; possibility of the permeation rate being too slow comparing to the dissolution one; the fact that the membranes cannot be used at acid pHs and thus shouldn't be used with gastric dissolution medium; the fact that it is a time consuming method. Therefore, these drawbacks invalidate the dialysis membranes method. As an alternative, ultracentrifugation, a simpler method, should be employed for free API quantification.

3.8. Prospects for modeling

Models capable of describing the disintegration from tablet to granules (developed for IR tablets) [87], [88], polymer swelling, erosion and API release (developed for extended release tablets) [89] processes are implemented in Dynochem (Scale-up Systems, Dublin, Ireland), a development and scale-up software for pharmaceutical industry. This software allows modifications to the models, but does not allow the implementation of other equations.

However, these models implemented in Dynochem are only suitable for crystalline APIs, i.e. its dissolution mechanism assumes an equilibrium solubility at the surface of the particles. Therefore, they are not suitable to describe the dissolution of amorphous solid dispersions whose dissolution is not limited by the API thermodynamic solubility. Even if one would define an amorphous “solubility”, i.e. the maximum increase in solution concentration that can be obtained relative to the crystalline form [90], this would not take into account the mass transfer within the polymeric matrix nor the swelling and erosion of the polymer.

In the developed model for amorphous APIs, it is assumed that the dissolution rate is governed by the mass transfer inside the polymeric matrix and therefore the API concentration at the surface of the dissolving particle is obtained by solving Fick’s law inside the particle with a boundary condition matching the mass flux of API entering the dissolution media (given by a Noyes-Whitney type equation). This is in stark contrast to the standard approach of decoupled dissolution dynamics by assuming that the API at the surface of the particles is at equilibrium concentration. As Dynochem does not allow the edition of the implemented code, it is not possible to link the modules of the Figure. Hence, the aforementioned adaptation, dissolution limited by API internal diffusion in spherical coordinates was implemented in Scilab, which has the additional benefit of being a free software.

The implemented work in this thesis is only a part of the overall model and its integration with the remaining modules has yet to be implemented in order to compare it to experimental dissolution data.

3.8.1. Model implementation

Fick’s second law of diffusion is used to describe the API mass transfer process. Numerical solutions of the respective set of partial differential equations are provided, considering radial diffusion within water-impermeable (there is no diffusion of the dissolution medium into the particle) spherical particles. Thus, they were solved numerically, using finite differences (Forward Euler scheme in time and second order scheme in space).

The particle radius varies with time due to the flux of the drug molecules from the particle surface. Correspondingly, the particle surface area reduces in time with decreasing particle radius and increasing bulk concentration.

The equations regarding API internal diffusion within the spherical polymeric matrix and its dissolution based in Wang et al. work [91] are presented hereafter. The main modification of the model from Wang et al. is the assumption that the surface particle is not saturated, but instead that it results from the mass balance of API diffusing from within the particle and the API leaving the particle to the dissolution medium.

API mass flux from an API particle into solution

One of the classic models regarding dissolution, the Noyes Whitney equation (Equation 1) considers K=A.D/ δ.V as a model constant. This is equivalent to assume that the diffusion layer thickness δ(t) decreases in time proportionally to the particle area A at all times. There is no basis for such an assumption. Wang et al. introduced the Sherwood (Sh) number into the model, where Sh is defined as the ratio of the characteristic rate for molecules to leave the surface and diffuse into the bulk to the characteristic rate for molecules to diffuse within the fluid over a distance with the same order of magnitude as the particle radius. Therefore, the mass flux of API leaving the particle to the surrounding liquid is given by:

$$J(t) = Sh\frac{D\left(C_{r=R(t)} - C_b(t)\right)}{R(t)} \quad \textbf{(11)}$$

$$Sh = \frac{R(t)}{\delta(t)} \quad \textbf{(12)}$$

J(t): API mass flux ($mg.mm^{-2}.s^{-1}$);
$C_{r=R}$: API concentration on particle surface ($mg.mm^{-3}$);
D: API diffusion coefficient in the bulk fluid ($mm^2.s^{-1}$);
r: radius (mm);
R(t): particle radius (mm);
C_b: API bulk concentration ($mg.mm^{-3}$);
Sh: Sherwood number;
δ (t): diffusion layer thickness (mm);
t: dissolution time (s).

In equilibrium, the two rates mentioned are comparable, i.e., the Sh number takes the value of 1:

$$\mathrm{J(t)} = \frac{\mathrm{D}\left(\mathrm{C_{r=R(t)}} - \mathrm{C_b(t)}\right)}{\mathrm{R(t)}} \quad \textbf{(13)}$$

API particle radius

Particle radius is reduced as the API molecules leave the particle. In the beginning of the dissolution test, particle radius is maximum and equivalent to the particle size of the particles tested.

$$\frac{\partial R(t)}{\partial t} = -{\rho_{DF}}^{-1} J(t) \quad \textbf{(14)}$$

Initial Condition:

$$t = 0, R(t) = R_{max} \quad (15)$$

R_{max}: initial particle radius (mm);
ρ_{DF}: API initial concentration within the particle, i.e. the ration between API mass and particle volume ($mg.mm^{-3}$).

Number of API particles in the system

$$N_p = \frac{m_{DF}}{\rho_{DF} \frac{4}{3} \pi R_{max}^3} \quad (16)$$

N_p: number of dissolving particles;
m_{DF}: mass of dosage form tested (mg);
R_{max}: initial particle radius (mm);

Surface area exposed to the dissolution medium

The area exposed and available to dissolution decreases with time as occurs to the particle radius. In the beginning of the dissolution test, surface area exposed is maximum.

$$A = 4\pi R(t)^2 N_p \quad (17)$$

Initial Condition:

$$t = 0, A = 4\pi {R_{max}}^2 N_p \quad (18)$$

A: Surface area exposed to the dissolution medium (mm^2).

Internal diffusion - 2nd Fick's Law:

In the beginning of the dissolution test, the API distribution within the particle is uniform and it corresponds to the API concentration.

Initial condition:

$$t = 0, C(0 < r < R_{max}) = \rho_{DF} \quad (19)$$

Boundary Conditions

- Symmetry on the left boundary:

$$\left.\frac{\partial C}{\partial r}\right|_{r=0} = 0 \quad (20)$$

- API Mass Flux on the right boundary:

$$\left.\frac{\partial C}{\partial r}\right|_{r=R(t)} = -\frac{D\left(C_{r=R(t)} - C_b(t)\right)}{R(t)} = -\frac{J(t)}{D} \quad \textbf{(21)}$$

Governing equation:

$$\frac{\partial C}{\partial t} = D\left(\frac{\partial^2 C}{\partial r^2} + \frac{2}{r}\frac{\partial C}{\partial r}\right) \quad \textbf{(22)}$$

C: API concentration within the particle ($mg.mm^{-3}$);
r: radius (mm).

Bulk concentration

The bulk fluid volume remains constant during dissolution. Dissolution experiments measure the increase in bulk concentration of the API with time, which is given by:

$$\frac{\partial C_b}{\partial t} = \frac{1}{V_b} J(t) A \quad \textbf{(23)}$$

V_b: bulk volume (mm^3).

3.8.2. Preliminary Results

The model was run for Formulation 2 – 600 μm. The granule size used as input was taken as the d50 mass: 250 μm. The API diffusion coefficient in the bulk fluid applied was chosen by trial and error, in order to adjust the theoretical data to the experimental data ($5x10^{-5}$ $mm^2.s^{-1}$). Three time and space instances were chosen arbitrarily. Figures 3.33, 3.34 and 3.35 show its output: concentration profiles within the particle and in the bulk fluid (dissolution test output).

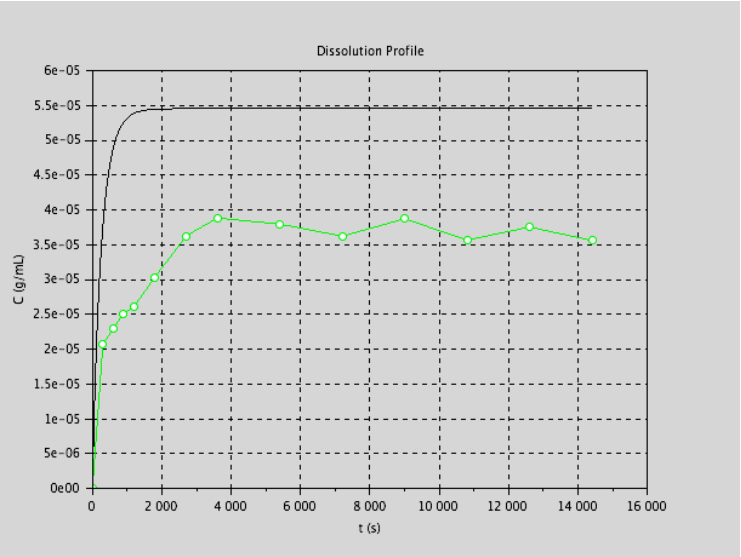


Figure 3.33 – Dissolution profiles of Formulation 2 – 600 μm: theoretical (black) and experimental (green).

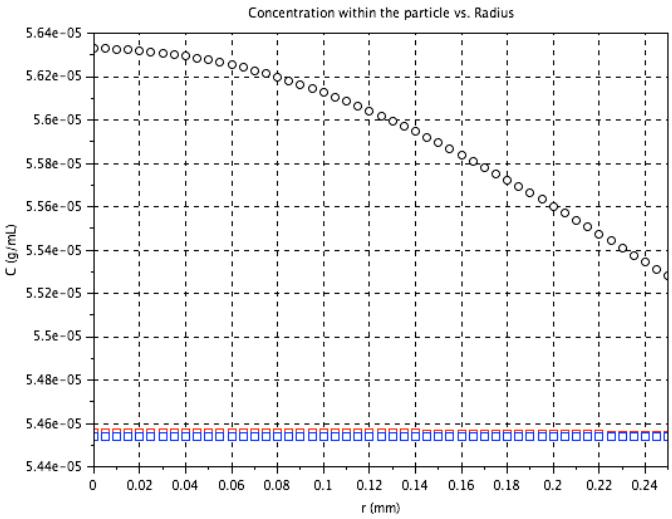


Figure 3.34 – Concentration within the particle vs Particle radius predicted by the dissolution model for Formulation 2 – 600 μm for three time instances: 2520 s (black), 5041 s (red) and 10082 s (blue).

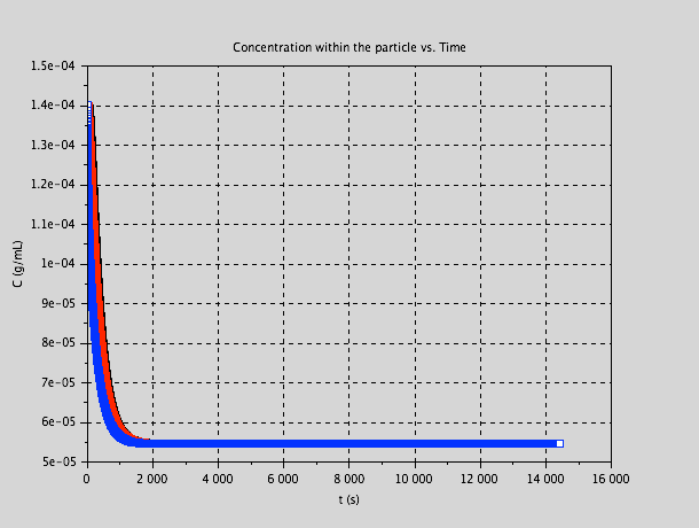


Figure 3.35 – Concentration within the particle vs Time for Formulation 2 – 600 μm predicted by the dissolution model for three space instances: 0.25 mm (black), 0.12 mm (red) and 0.25 mm (blue).

The results of the simulations of the dissolution profiles of the different granule sizes of formulation 2 (212 μm, 600 μm and 800 μm), with the d50 of 45 μm, 250 μm and 350 μm, respectively are presented in Figure 3.5.

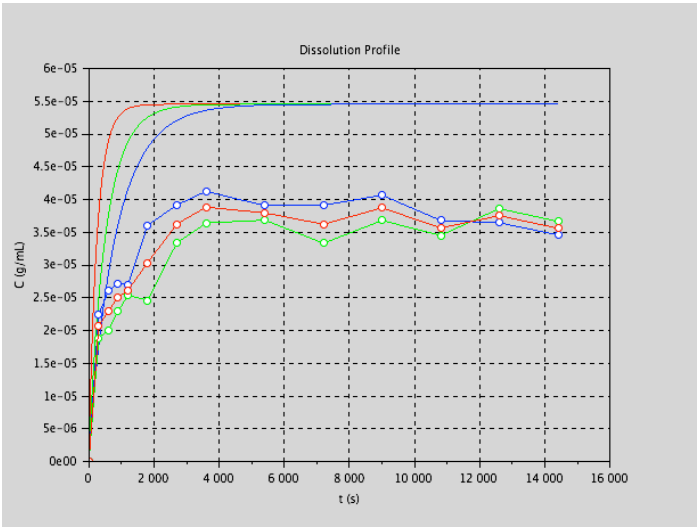


Figure 3.36 – Dissolution profiles of Formulation 2 – 212 μm (red), Formulation 2 - 600 μm (green) and Formulation 2 - 800 μm (blue) predicted by the model. The respective experimental profiles are represented by the lines of same colors with circle markers.

4

Conclusions and Future Work

The results of the current study suggest that solid molecular dispersions of ITZ prepared by the spray drying method can be used to enhance the apparent solubility and subsequent dissolution rate of this poorly soluble drug.

This work shows the importance of dissolution testing in environments physiologically relevant during formulation development. Biorelevant dissolution methodology demonstrated considerable discriminative power to evaluate different formulations and inclusively, different particle sizes. It also demonstrated that simple aqueous buffers are not enough to predict and evaluate dosage forms performance because they can often lead to a sub or over estimation of the solubility *in vivo*. Additionally, the potential for higher bioavailability of solid oral ITZ via intestinal targeting was demonstrated.

In conclusion, modern formulation development should rely on biorelevant test methods, which can ally discriminative to predictive power, since they can enable an early identification of potential problems in drug release rate and hasten the formulation process as they are a useful tool in the formulation development process, particularly when the rate limiting step to absorption is the dissolution. Moreover, coupling these tests with *in silico* tools offers the potential to reduce the number of *in vivo* an even *in vitro* tests, thus speeding the screening and development process and making it more cost-effective. Therefore, robust and reliable tests for the characterization of the release patterns in each step of formulation, from the API to the newly formulated immediate release or modified-release products are an indispensable tool in the pharmaceutical and process development scientist's toolbox.

For further development of this work, studies on stability testing of the SDDs should be conducted. The correlation between storage condition and the duration of physical stability of solid molecular dispersions should be studied and completed for future references. Additionally, the disintegrant percentage should be adjusted in the tablet formulation. Moreover, values of

pharmacokinetic parameters (i.e. AUC infinity, C_{max}, T_{max} and $t_{1/2}$) should be obtained to demonstrate the improvement of blood concentration profile and BA with these stabilized amorphous ITZ systems and an *in vitro in vivo* correlation (IVIVC) of ITZ solid molecular dispersions should be investigated in animal models.

The dialysis membrane/ultracentrifugation methods, even though enabling free drug quantification, are not necessarily biorelevant. Consequently, further studies with cellular models, e.g. Caco 2 cells should be conducted. These tests allow the discrimination of several transport mechanisms, inclusively, using biorelevant media. With these permeability tests it would be possible to study the effect of excipients that modify drug permeability, such as d-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) and for example solubility enhancers that often reduce drugs permeability.

It is also proposed for future work API, SDI and tablet dissolution testing in USP Apparatus 4. As previously stated, the USP Apparatus 4 has the advantage of maintenance of sink conditions throughout dissolution tests, the possibility of dissolution media change and of enabling suitable dissolution tests for powders. Its hydrodynamic conditions, approximated to the *in vivo* ones would enable the stablishing of an IVIVC.

For further development of dissolution tests cheaper simplified biorelevant media corresponding to buffers with surfactants existing *in vivo* should be studied and it would be also important to develop miniaturized dissolution tests, that use very small quantities of API and media that could be useful for formulation screening.

A good follow-up for the developed *in silico* work is the upgrading of this model for describing the polymer swelling as a result of the diffusion of the bulk fluid (permeable particles) and the dependence of the diffusion coefficient of the species with the bulk fluid concentration. Lastly, the following steps could be developed: the linkage of the different modules, i.e. disintegration from tablet to granules (implemented in Dynochem), API release (implemented in Dynochem and adapted to amorphous APIs in this work), supported by experiences designed to isolate the different effects and the building of a user-friendly interface. Also, in order to describe the “spring and parachute” effect, a precipitation model should be implemented and linked in the future.

5

References

[1] S. T. Buckley, S. M. Fischer, G. Fricker, and M. Brandl, “In vitro models to evaluate the permeability of poorly soluble drug entities: Challenges and perspectives,” *Eur. J. Pharm. Sci.*, vol. 45, no. 3, pp. 235–250, Feb. 2012.

[2] A. C. F. Rumondor, S. S. Dhareshwar, and F. Kesisoglou, “Amorphous Solid Dispersions or Prodrugs: Complementary Strategies to Increase Drug Absorption,” *J. Pharm. Sci.*, vol. 105, no. 9, pp. 2498–2508, Jan. 2016.

[3] B. Griffin and C. O. Driscoll, *Drug Absorption Studies*, vol. VII. Boston, MA: Springer US, 2008.

[4] P. S. Klein, “Biorelevant Dissolution: Concepts and Application,” 2011. [Online]. Available: http://www.aaps.org/uploadedFiles/Content/Sections_and_Groups/Focus_Groups/In_Vitro_Release_and_Dissolution_Testing/Resources/IVRDTFGKlein2011.pdf. [Accessed: 01-Apr-2015].

[5] J. M. Cardot and I. Tomic, “In vitro in vivo correlation basis and application to slow release injectable formulation,” *Farmacia*, vol. 63, no. 6, pp. 781–791, 2015.

[6] Q. Wang, N. Fotaki, and Y. Mao, “Biorelevant Dissolution: Methodology and Application in Drug Development,” *Dissolution Technol.*, vol. 16, no. 3, pp. 6–12, 2009.

[7] E. Scheubel, “Predictive in vitro dissolution tools : application during formulation development,” Universit´e d’Auvergne - Clermont-Ferrand I, 2010.

[8] C. W. Pouton, “Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system,” *Eur. J. Pharm. Sci.*, vol. 29, no. 3–4 SPEC. ISS., pp. 278–287, Nov. 2006.

[9] J. M. Butler and J. B. Dressman, “The developability classification system: Application of biopharmaceutics concepts to formulation development,” *J. Pharm. Sci.*, vol. 99, no. 12, pp. 4940–4954, Dec. 2010.

[10] L. Z. Benet, C.-Y. Wu, and J. M. Custodio, “Predicting drug absorption and the effects of food on oral bioavailability,” *Bull. Tech. Gattefossé 2006 - 40th Anniv. Influ. lipid excipients oral drug Absorpt.*, vol. 99, pp. 9–16, 2006.

[11] R. Tiwari, G. Tiwari, B. Srivastava, and A. K. Rai, “Solid dispersions: An overview to modify bioavailability of poorly water soluble drugs,” *Int. J. PharmTech Res.*, vol. 1, no. 4, pp. 1338–1349, 2009.

[12] N. Fotaki and M. Vertzoni, “Biorelevant dissolution methods and their applications in in vitro- in vivo correlations for oral formulations,” *Open Drug Deliv. J.*, vol. 4, no. 2, pp. 2–13, 2010.

[13] E. Galia, E. Nicolaides, D. Hörter, R. Löbenberg, C. Reppas, and J. B. Dressman, “Evaluation of Various Dissolution Media for Predicting In Vivo Performance of Class I and II Drugs,” *Pharm. Res.*, vol. 15, no. 5, pp. 698–705, 1998.

[14] R. Löbenberg, J. Krämer, V. P. Shah, G. L. Amidon, and J. B. Dressman, “Dissolution Testing as a Prognostic Tool for Oral Drug Absorption: Dissolution Behavior of Glibenclamide,” *Pharm. Res.*, vol. 17, no. 4, pp. 439–444, Apr. 2000.
[15] K. Kleberg, J. Jacobsen, and A. Müllertz, “Characterising the behaviour of poorly water soluble drugs in the intestine: application of biorelevant media for solubility, dissolution and transport studies,” *J. Pharm. Pharmacol.*, vol. 62, no. 11, pp. 1656–1668, Nov. 2010.
[16] A. A. Noyes and W. R. Whitney, “THE RATE OF SOLUTION OF SOLID SUBSTANCES IN THEIR OWN SOLUTIONS.,” *J. Am. Chem. Soc.*, vol. 19, no. 12, pp. 930–934, Dec. 1897.
[17] E. Nicolaides, E. Galia, C. Efthymiopoulos, J. B. Dressman, and C. Reppas, “Forecasting the In Vivo Performance of Four Low Solubility Drugs from Their In Vitro Dissolution Data,” *Pharm. Res.*, vol. 16, no. 12, pp. 1876–1882, Dec. 1999.
[18] J. B. Dressman, G. L. Amidon, C. Reppas, and V. P. Shah, “Dissolution testing as a prognostic tool for oral drug absorption: Immediate release dosage forms,” *Pharm. Res.*, vol. 15, no. 1, pp. 11–22, Jan. 1998.
[19] D. M. Mudie, G. L. Amidon, and G. E. Amidon, “Physiological parameters for oral delivery and in vitro testing,” *Mol. Pharm.*, vol. 7, no. 5, pp. 1388–1405, Oct. 2010.
[20] L. Shargel, S. Wu-Pong, and A. B. C. Yu, “Applied Biopharmaceutics & Pharmacokinetics,” 6th ed., New York: McGraw-Hill Medical, 2012, p. 768.
[21] R. Bettini, “Pharmaceutical dissolution testing,” *J. Control. Release*, vol. 32, no. 2, pp. 204–205, Dec. 1994.
[22] S. Klein, “The Use of Biorelevant Dissolution Media to Forecast the In Vivo Performance of a Drug,” *AAPS J.*, vol. 12, no. 3, pp. 397–406, Sep. 2010.
[23] B. Steffansen, B. Brodin, and C. Uhd Nielsen, *Molecular biopharmaceutics : aspects of drug characterisation, drug delivery and dosage form evaluation*, 1st ed. London: Pharmaceutical Press, 2010.
[24] E. Galia, J. Horton, and J. B. Dressman, “Albendazole Generics—A Comparative In Vitro Study,” *Pharm. Res.*, vol. 16, no. 12, pp. 1871–1875, 1999.
[25] P. Luner, “Wetting characteristics of media emulating gastric fluids,” *Int. J. Pharm.*, vol. 212, no. 1, pp. 81–91, Jan. 2001.
[26] M. Vertzoni, E. Pastelli, D. Psachoulias, L. Kalantzi, and C. Reppas, “Estimation of Intragastric Solubility of Drugs: In What Medium?,” *Pharm. Res.*, vol. 24, no. 5, pp. 909–917, Apr. 2007.
[27] M. Vertzoni, J. Dressman, J. Butler, J. Hempenstall, and C. Reppas, “Simulation of fasting gastric conditions and its importance for the in vivo dissolution of lipophilic compounds,” *Eur. J. Pharm. Biopharm.*, vol. 60, no. 3, pp. 413–417, Aug. 2005.
[28] J. B. Dressman, R. R. Berardi, L. C. Dermentzoglou, T. L. Russell, S. P. Schmaltz, J. L. Barnett, and K. M. Jarvenpaa, “Upper Gastrointestinal (GI) pH in Young, Healthy Men and Women,” *Pharm. Res.*, vol. 7, no. 7, pp. 756–761, 1990.
[29] D. Hörter and J. B. Dressman, “Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract,” *Adv. Drug Deliv. Rev.*, vol. 25, no. 1, pp. 3–14, 1997.
[30] M. Efentakis and J. B. Dressman, “Gastric juice as a dissolution medium: Surface tension and pH,” *Eur. J. Drug Metab. Pharmacokinet.*, vol. 23, no. 2, pp. 97–102, Jun. 1998.
[31] L. Kalantzi, K. Goumas, V. Kalioras, B. Abrahamsson, J. B. Dressman, and C. Reppas, “Characterization of the Human Upper Gastrointestinal Contents Under Conditions Simulating Bioavailability/Bioequivalence Studies,” *Pharm. Res.*, vol. 23, no. 1, pp. 165–176, Jan. 2006.
[32] S. Klein, J. B. Dressman, J. Butler, J. M. Hempenstall, and C. Reppas, “Media to simulate the postprandial stomach I. Matching the physicochemical characteristics of standard breakfasts,” *J. Pharm. Pharmacol.*, vol. 56, no. 5, pp. 605–610, May 2004.
[33] P. Macheras, M. Koupparis, and C. Tsaprounis, “Drug dissolution studies in milk using the automated flow injection serial dynamic dialysis technique,” *Int. J. Pharm.*, vol. 33, no. 1–3, pp. 125–136, Nov. 1986.
[34] E. Jantratid, N. Janssen, C. Reppas, and J. B. Dressman, “Dissolution Media Simulating Conditions in the Proximal Human Gastrointestinal Tract: An Update,” *Pharm. Res.*, vol. 25, no. 7, pp. 1663–1676, Jul. 2008.
[35] N. Fotaki and M. Vertzoni, “Biorelevant Dissolution Methods and Their Applications in In Vitro- In Vivo Correlations for Oral Formulations,” *Open Drug Deliv. J.*, vol. 4, no. 2, pp. 2–

13, May 2010.
[36] L. Kalantzi, E. Persson, B. Polentarutti, B. Abrahamsson, K. Goumas, J. B. Dressman, and C. Reppas, “Canine Intestinal Contents vs. Simulated Media for the Assessment of Solubility of Two Weak Bases in the Human Small Intestinal Contents,” *Pharm. Res.*, vol. 23, no. 6, pp. 1373–1381, Jun. 2006.
[37] K. Otsuka, Y. Shono, and J. Dressman, *Coupling biorelevant dissolution methods with physiologically based pharmacokinetic modelling to forecast in-vivo performance of solid oral dosage forms*, 1st ed., vol. 65, no. 7. Shaker Verlag, 2013.
[38] J. H. Wood, J. E. Syarto, and H. Letterman, “Improved holder for intrinsic dissolution rate studies,” *J. Pharm. Sci.*, vol. 54, no. 7, pp. 1068–1068, Jul. 1965.
[39] *<1087> INTRINSIC DISSOLUTION*. Rockville Maryland, USA: U.S. Phamacopoeia, 2013.
[40] M. G. Issa and H. G. Ferraz, “Intrinsic dissolution as a tool for evaluating drug solubility in accordance with the biopharmaceutics classification system,” *Dissolution Technol.*, vol. 18, no. 3, pp. 6–13, 2011.
[41] R. Uddin, N. Saffoon, and K. B. Sutradhar, “Dissolution and Dissolution Apparatus : A Review,” *Int. J. Curr. Biomed. Pharm. Res.*, vol. 1, no. 4, pp. 201–207, 2011.
[42] M. Pernarowski, W. Woo, and R. O. Searl, “Continuous flow apparatus for the determination of the dissolution characteristics of tablets and capsules,” *J. Pharm. Sci.*, vol. 57, no. 8, pp. 1419–1421, Aug. 1968.
[43] E. Stippler, “Compendial Dissolution: Theory and Practice,” 2011. [Online]. Available: http://www2.aaps.org/uploadedFiles/Content/Sections_and_Groups/Focus_Groups/In_Vitro_Release_and_Dissolution_Testing/Resources/IVRDTFGStippler2011.pdf. [Accessed: 01-Mar-2016].
[44] E. S. Kostewicz, B. Abrahamsson, M. Brewster, J. Brouwers, J. Butler, S. Carlert, P. A. Dickinson, J. Dressman, R. Holm, S. Klein, J. Mann, M. McAllister, M. Minekus, U. Muenster, A. Müllertz, M. Verwei, M. Vertzoni, W. Weitschies, and P. Augustijns, “In vitro models for the prediction of in vivo performance of oral dosage forms,” *Eur. J. Pharm. Sci.*, vol. 57, no. 1, pp. 342–366, Jun. 2014.
[45] A. Kambayashi, T. Yasuji, and J. B. Dressman, “Prediction of the precipitation profiles of weak base drugs in the small intestine using a simplified transfer (‘dumping’) model coupled with in silico modeling and simulation approach,” *Eur. J. Pharm. Biopharm.*, vol. 103, pp. 95–103, Jun. 2016.
[46] E. S. Kostewicz, M. Wunderlich, U. Brauns, R. Becker, T. Bock, and J. B. Dressman, “Predicting the precipitation of poorly soluble weak bases upon entry in the small intestine,” *J. Pharm. Pharmacol.*, vol. 56, no. 1, pp. 43–51, Jan. 2004.
[47] I. Borst, S. Ugwu, and A. H. Beckett, “New and Extended Applications for USP Drug Release Apparatus 3.,” *Dissolution Technol.*, vol. 4, no. 1, pp. 11–18, 1997.
[48] J. Gajendran, J. Krämer, and L. Timothy Grady, “Historical Development of Dissolution Testing,” in *Pharmaceutical Dissolution Testing*, Informa Healthcare, 2005, pp. 1–37.
[49] S. Missaghi and R. Fassihi, “Release characterization of dimenhydrinate from an eroding and swelling matrix: selection of appropriate dissolution apparatus,” *Int. J. Pharm.*, vol. 293, no. 1–2, pp. 35–42, Apr. 2005.
[50] N. Fotaki, “Flow-Through Cell Apparatus (USP Apparatus 4): Operation and Features,” *Dissolution Technol.*, vol. 18, no. 4, pp. 46–49, 2011.
[51] A. Paprskářová, P. Možná, E. F. Oga, A. Elhissi, and M. A. Alhnan, “Instrumentation of Flow-Through USP IV Dissolution Apparatus to Assess Poorly Soluble Basic Drug Products: a Technical Note,” *AAPS PharmSciTech*, vol. 17, no. 5, pp. 1261–1266, Oct. 2016.
[52] I. Singh and H. Y. Aboul-Enein, “Advantages of USP Apparatus IV (flow-through cell apparatus) in dissolution studies,” *J. Iran. Chem. Soc.*, vol. 3, no. 3, pp. 220–222, Sep. 2006.
[53] N. Fotaki and C. Reppas, “The flow through cell methodology in the evaluation of intralumenal drug release characteristics,” *Dissolution Technol.*, vol. 12, no. 2, pp. 17–21, 2005.
[54] E. Stippler, “Compendial Dissolution: Theory and Practice,” 2011.
[55] H. O. Ammar, S. A. El-Nahhas, L. H. Emara, M. M. Ghorab, and H. A. Salama, “Discrepancy among dissolution rates of commercial tablets as a function of the dissolution method. Part 7: Aspirin,” *Pharmazie*, vol. 52, no. 2, pp. 145–149, Dec. 1997.

[56] W. C. G. Butler and S. R. Bateman, “A flow-through dissolution method for a two component drug formulation where the actives have markedly differing solubility properties,” *Int. J. Pharm.*, vol. 173, no. 1–2, pp. 211–219, Oct. 1998.
[57] M. S. Bloomfield and W. C. Butler, “Robustness testing, using experimental design, of a flow-through dissolution method for a product where the actives have markedly differing solubility properties,” *Int. J. Pharm.*, vol. 206, no. 1–2, pp. 55–61, Sep. 2000.
[58] K. De Beule, “Itraconazole: Pharmacology, clinical experience and future development,” *Int. J. Antimicrob. Agents*, vol. 6, no. 3, pp. 175–181, 1996.
[59] T. Parikh, H. K. Sandhu, T. T. Talele, and A. T. M. Serajuddin, “Characterization of Solid Dispersion of Itraconazole Prepared by Solubilization in Concentrated Aqueous Solutions of Weak Organic Acids and Drying,” *Pharm. Res.*, vol. 33, no. 6, pp. 1456–1471, Jun. 2016.
[60] H. G. Brittain, *Polymorphism in Pharmaceutical Solids*, 2nd ed. New York: CRC Press, 2009.
[61] J. A. Barone, J. G. Koh, R. H. Bierman, J. L. Colaizzi, K. A. Swanson, M. C. Gaffar, B. L. Moskovitz, W. Mechlinski, and V. Van de Velde, “Food interaction and steady-state pharmacokinetics of itraconazole capsules in healthy male volunteers.,” *Antimicrob. Agents Chemother.*, vol. 37, no. 4, pp. 778–784, Apr. 1993.
[62] D. T. Friesen, R. Shanker, M. Crew, D. T. Smithey, W. J. Curatolo, and J. A. S. Nightingale, “Hydroxypropyl methylcellulose acetate succinate-based spray-dried dispersions: An overview,” *Mol. Pharm.*, vol. 5, no. 6, pp. 1003–1019, 2008.
[63] Y. He and C. Ho, “Amorphous Solid Dispersions: Utilization and Challenges in Drug Discovery and Development,” *J. Pharm. Sci.*, vol. 104, no. 10, pp. 3237–3258, Oct. 2015.
[64] K. Nidhi, S. Indrajeet, M. Khushboo, K. Gauri, and D. J. Sen, “Hydrotropy: A promising tool for solubility enhancement: A review,” *Int. J. Drug Dev. Res.*, vol. 3, no. 2, pp. 26–33, 2011.
[65] S. M. Fischer, M. Brandl, and G. Fricker, “Effect of the non-ionic surfactant Poloxamer 188 on passive permeability of poorly soluble drugs across Caco-2 cell monolayers,” *Eur. J. Pharm. Biopharm.*, vol. 79, no. 2, pp. 416–422, Oct. 2011.
[66] K. J. Frank, U. Westedt, K. M. Rosenblatt, P. Hölig, J. Rosenberg, M. Mägerlein, M. Brandl, and G. Fricker, “Impact of FaSSIF on the solubility and dissolution/permeation rate of a poorly water-soluble compound,” *Eur. J. Pharm. Sci.*, vol. 47, no. 1, pp. 16–20, 2012.
[67] K. J. Frank, K. M. Rosenblatt, U. Westedt, P. Hölig, J. Rosenberg, M. Mägerlein, G. Fricker, and M. Brandl, “Amorphous solid dispersion enhances permeation of poorly soluble ABT-102: True supersaturation vs. apparent solubility enhancement,” *Int. J. Pharm.*, vol. 437, no. 1–2, pp. 288–293, 2012.
[68] G. A. Ilevbare and L. S. Taylor, “Liquid–Liquid Phase Separation in Highly Supersaturated Aqueous Solutions of Poorly Water-Soluble Drugs: Implications for Solubility Enhancing Formulations,” *Cryst. Growth Des.*, vol. 13, no. 4, pp. 1497–1509, Apr. 2013.
[69] Y. L. Hsieh, G. A. Ilevbare, B. Van Eerdenbrugh, K. J. Box, M. V. Sanchez-Felix, and L. S. Taylor, “pH-Induced Precipitation Behavior of Weakly Basic Compounds: Determination of Extent and Duration of Supersaturation Using Potentiometric Titration and Correlation to Solid State Properties,” *Pharm. Res.*, vol. 29, no. 10, pp. 2738–2753, Oct. 2012.
[70] Í. Duarte, J. L. Santos, J. F. Pinto, and M. Temtem, “Screening methodologies for the development of spray-dried amorphous solid dispersions,” *Pharm. Res.*, vol. 32, no. 1, pp. 222–237, 2015.
[71] “Biotech Grade Dialysis Membranes - Cellulose Ester (CE), RegeneratedCellulose (RC).” [Online]. Available: http://spectrumlabs.com/lit/420x10688x000.pdf. [Accessed: 01-Sep-2016].
[72] H. S. Ghazal, A. M. Dyas, J. L. Ford, and G. A. Hutcheon, “In vitro evaluation of the dissolution behaviour of itraconazole in bio-relevant media,” *Int. J. Pharm.*, vol. 366, no. 1–2, pp. 117–123, Jan. 2009.
[73] R. Bettini and D. Cocconi, “Handbook of Pharmaceutical Excipients, Third Edition,” *J. Control. Release*, vol. 71, no. 3, pp. 352–353, Apr. 2001.
[74] S. Zhang, T. W. Y. Lee, and A. H. L. Chow, “Crystallization of Itraconazole Polymorphs from Melt,” *Cryst. Growth Des.*, vol. 16, no. 7, pp. 3791–3801, Jul. 2016.
[75] K. Six, G. Verreck, J. Peeters, K. Binnemans, H. Berghmans, P. Augustijns, R. Kinget, and G. Van den Mooter, “Investigation of thermal properties of glassy itraconazole: Identification of a monotropic mesophase,” *Thermochim. Acta*, vol. 376, no. 2, pp. 175–

181, 2001.
[76] B. C. Hancock and G. Zografi, “The Relationship Between the Glass Transition Temperature and the Water Content of Amorphous Pharmaceutical Solids,” *Pharm. Res.*, vol. 11, no. 4, pp. 471–477, 1994.
[77] D. Ouyang and S. C. Smith, *Computational Pharmaceutics: Application of Molecular Modeling in Drug Delivery*, 1st ed. Sussex: John Wiley & Sons, Inc., 2015.
[78] R. Vehring, “Pharmaceutical Particle Engineering via Spray Drying,” *Pharm. Res.*, vol. 25, no. 5, pp. 999–1022, May 2008.
[79] A. Newman, *Pharmaceutical Amorphous Solid Dispersions*, 1st ed. New Jersey: John Wiley & Sons, Inc., 2015.
[80] S. D. Mithani, V. Bakatselou, C. N. TenHoor, and J. B. Dressman, “Estimation of the Increase in Solubility of Drugs as a Function of Bile Salt Concentration,” *Pharm. Res.*, vol. 13, no. 1, pp. 163–167, 1996.
[81] W. Curatolo, J. A. Nightingale, and S. M. Herbig, “Utility of Hydroxypropylmethylcellulose Acetate Succinate (HPMCAS) for Initiation and Maintenance of Drug Supersaturation in the GI Milieu,” *Pharm. Res.*, vol. 26, no. 6, pp. 1419–1431, Jun. 2009.
[82] “Sporanox® Data Sheet,” 2016. [Online]. Available: http://www.medsafe.govt.nz/Profs/Datasheet/s/sporanoxcap.pdf. [Accessed: 01-Aug-2016].
[83] J. Thiry, G. Broze, A. Pestieau, A. S. Tatton, F. Baumans, C. Damblon, F. Krier, and B. Evrard, “Investigation of a suitable in vitro dissolution test for itraconazole-based solid dispersions,” *Eur. J. Pharm. Sci.*, vol. 85, pp. 94–105, Mar. 2016.
[84] D. A. Miller, J. C. DiNunzio, W. Yang, J. W. McGinity, and R. O. Williams, “Targeted intestinal delivery of supersaturated itraconazole for improved oral absorption,” *Pharm. Res.*, vol. 25, no. 6, pp. 1450–1459, Jun. 2008.
[85] S. T. Buckley, K. J. Frank, G. Fricker, and M. Brandl, “Biopharmaceutical classification of poorly soluble drugs with respect to ‘enabling formulations,’” *Eur. J. Pharm. Sci.*, vol. 50, no. 1, pp. 8–16, Sep. 2013.
[86] X. Xu, M. A. Khan, and D. J. Burgess, “A two-stage reverse dialysis in vitro dissolution testing method for passive targeted liposomes,” *Int. J. Pharm.*, vol. 426, no. 1–2, pp. 211–218, Apr. 2012.
[87] J. Siepmann, “Calculation of the required size and shape of hydroxypropyl methylcellulose matrices to achieve desired drug release profiles,” *Int. J. Pharm.*, vol. 201, no. 2, pp. 151–164, May 2000.
[88] J. Siepmann, K. Podual, M. Sriwongjanya, N. A. Peppas, and R. Bodmeier, “A New Model Describing the Swelling and Drug Release Kinetics from Hydroxypropyl Methylcellulose Tablets,” *J. Pharm. Sci.*, vol. 88, no. 1, pp. 65–72, Jan. 1999.
[89] D. Wilson, S. Wren, and G. Reynolds, “Linking Dissolution to Disintegration in Immediate Release Tablets Using Image Analysis and a Population Balance Modelling Approach,” *Pharm. Res.*, vol. 29, no. 1, pp. 198–208, Jan. 2012.
[90] L. Almeida e Sousa, S. M. Reutzel-Edens, G. A. Stephenson, and L. S. Taylor, “Assessment of the amorphous ‘solubility’ of a group of diverse drugs using new experimental and theoretical approaches,” *Mol. Pharm.*, vol. 12, no. 2, pp. 484–495, 2015.
[91] Y. Wang, B. Abrahamsson, L. Lindfors, and J. G. Brasseur, “Comparison and analysis of theoretical models for diffusion-controlled dissolution,” *Mol. Pharm.*, vol. 9, no. 5, pp. 1052–1066, Apr. 2012.
[92] C. Roy, J. Chakrabarty, and H. B. Patel, “Development and Validation of a Stability Indicating Binary RP-UPLC Method for Determination of Itraconazole in Capsules dosage form,” vol. 2, no. 3, pp. 165–174, 2012.
[93] *〈1225〉 VALIDATION OF COMPENDIAL PROCEDURES*. Rockville Maryland, USA: U.S. Phamacopoeia, 2013.

Appendix 1 – Validation of HPLC method for ITZ quantification in SDD and tablets

HPLC method development

Based on a literature survey, a method was tested [92] with satisfactory results. The proportions of mobile phase composition were tested in order to obtain satisfactory retention factors. Optimal conditions were achieved using as mobile phase a mixture of acetonitrile, tetrahydrofuran (THF) and water adjusted to pH 2.5 with phosphoric acid (50:2.5:47.5) v/v, respectively. A flow rate of 0.4 mL min^{-1} gave an optimal signal-to-noise ratio and an appropriate separation time. The maximum absorption of the compound was 260 nm, the selected wavelength for analysis. The retention time of ITZ (Itraconazole) was approximately 3 minutes and the time required for analysis is 8 minutes. The HPLC method is described in Chapter 2 – Materials and Methods.

Preparation of the standard solutions

The stock solution (200 μg/mL) was prepared by dissolving 20 mg of ITZ in a 100 mL volumetric flask with the dissolution mixture (MeOH:H_2O 90:10 v/v). The standard solutions were prepared by dilution of the stock solution with the dissolution mixture to yield five different concentrations: 1, 5, 25, 50 and 75 μg/mL.

Linearity

Linearity was determined by calculation of a regression line from the peak area vs concentration plot for five standard solutions (1, 5, 25, 50 and 75 μg/mL) in triplicates (n=3). The slope and the square of the sample correlation coefficient of the calibration curve were calculated by linear regression, with the software Microsoft® Excel 2013.

ITZ was found to follow the Lambert-Beer law over the range of concentrations 1 to 75 µg/mL in triplicate (n=3) ($R^2 \geq 0.999$) and the relative standard deviation of three injections was less than 1% (Figure 6.1 and Table 6.1)

Table 6.1 – ITZ linearity data.

Theoretical concentration (µg/mL)	Average peak area (µV*s)	SD	RSD (%)
1	5758	20.5	0.4
5	28850	116.5	0.4
25	149384	278.0	0.2
50	149384	1022.5	0.3
75	435890	898.5	0.2

Selectivity

The selectivity of the chromatographic method was assessed with respective physical mixtures of the SDDs and tablets produced, with and without ITZ, in the same mixture of H_2O:MeOH (50:50 v/v). This study supports the specificity of the method, since no additional peaks to ITZ were observed.

Sensivity

The Limit Of Quantification (LOQ), the minimum level at which the API concentration can be determined with acceptable accuracy and precision, was considered as the lowest ITZ standard with a signal to noise ratio over to 10 [93]. The determined LOQ of the UPLC method was 1 µg/mL, with a signal to noise ratio of 10.

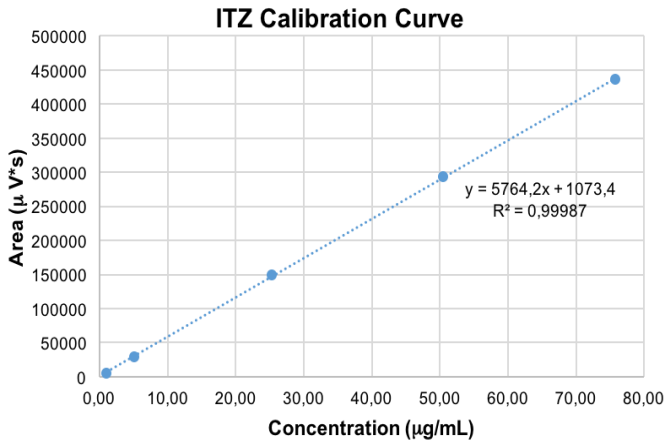


Figure 6.1 – ITZ linearity.

In order to complete this method validation, LOD (Limit Of Detection) must be determined and accuracy and precision shall be assessed.

Appendix 2 – Good Practices Regarding the Dissolution Method

The workflow for dissolution tests during formulation development stage is shown in Figure 8.1. The first step in drug product development is to classify the API in the BCS system in order to decide the formulation strategy and accordingly, design the future dissolution tests.

The solubility and permeability assessment can be made with a variety of dissolution media that must cover the pH range of the GI tract. This characterization will allow the choice of formulation strategy, since the goal is to achieve the behavior of a BCS class I drug.

After the API is formulated, it is important to develop dissolution methods that are discriminative enough to compare and optimize formulations. If the drug is not solubility-limited, simple buffers can be used as dissolution media for this purpose and test conditions can be changed in order to ensure this discriminative power. If the drug is solubility-limited, biorelevant media should be used.

If the absorption of the API is solubility limited, an IVIVC can be established and for that, predictive dissolution tests, i.e. biorelevant, are needed.

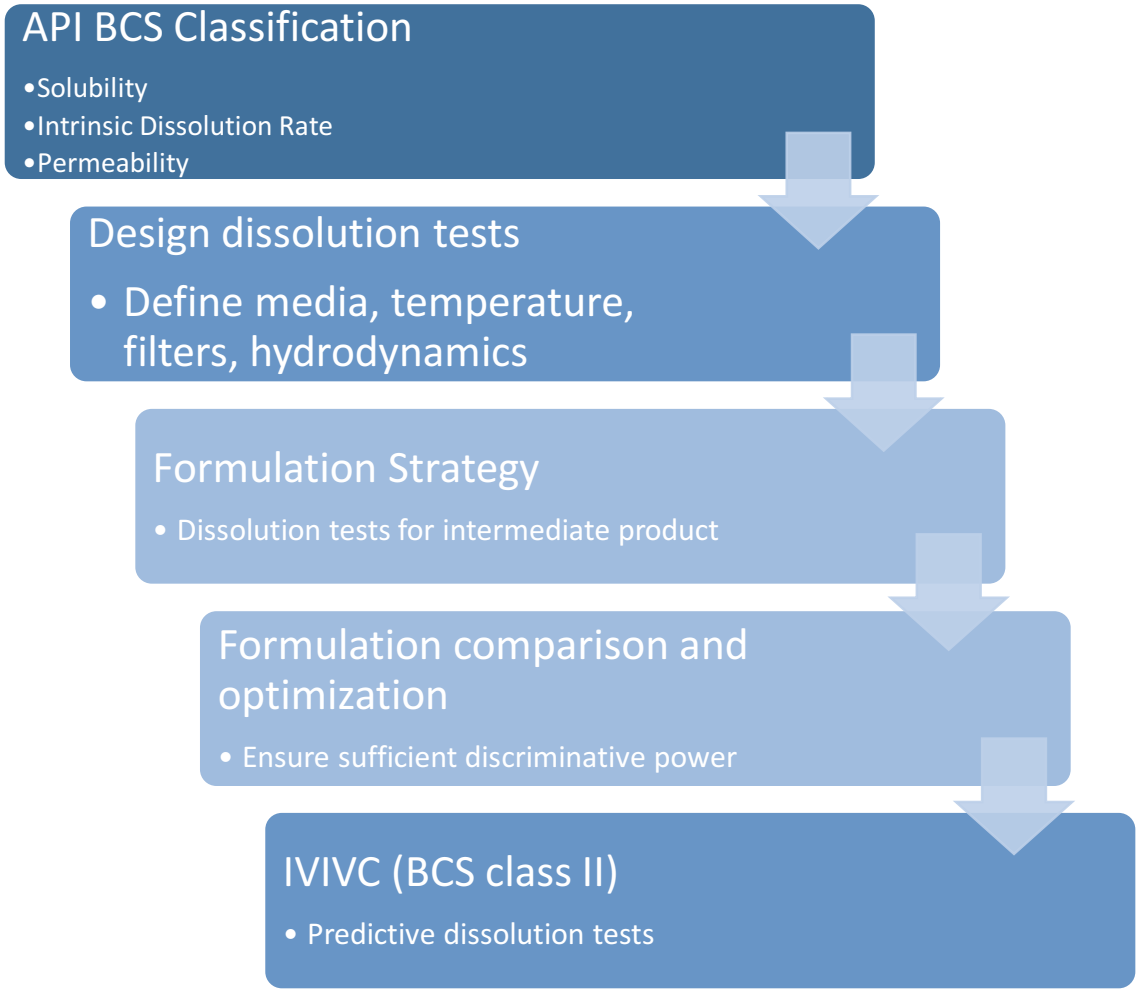


Figure 7.1 – Workflow for dissolution testing during formulation development stage.

This work highlighted some important aspects related to the dissolution testing method designed for drug development that sometimes are undervalued and constitute significant recommendations for future works, such as:

- Filters choice: the material must be selected so that the API dissolved is not retained by the filter. In this regard, filter compatibility tests must be performed before the design of the dissolution test;
- Complete knowledge of the dosage form: it is critical to know the physicochemical properties of the dosage form, in all the stages of the formulation process, namely its solubility in the media tested. This will allow to determine whether if the test is performed under sink or non sink conditions and to evaluate the dissolution results with this value, being limiting to dissolution or not. Also, if the API does not have low aqueous solubility, a compendial medium, representing the physiological pH range can be sufficient to guide the formulation process;
- Tests under non sink conditions: additional attention has to be taken during these tests; To prevent precipitation upon sampling, sample filtration and dilution with an

organic solvent must occur immediately (because the drug concentration in these tests rapidly achieves drug solubility and any small change in the conditions, i.e., temperature, will affect the equilibrium).