Mechanistic Dissolution Modeling of a Poorly Soluble Drug; an Evaluation of Formulation Influence and Simulation Parameters for Enhancing Predictive Capability

By

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Abstract

In early drug development, the selection of a formulation platform and decisions on formulation strategies have to be made within a short timeframe and often with minimal use of the active pharmaceutical ingredient (API). At this stage, there is limited information available about the physicochemical and biopharmaceutical properties of a new drug candidate. The current work evaluated the various physicochemical parameters required to improve dissolution profile prediction accuracy at the early stage of drug development and estimate the effect of formulation strategies on the dissolution profile of immediate release tablets of a poorly soluble drug using in silico tools.

In the first study, DDDPlus™ (Dose Disintegration and Dissolution Plus) was used in simulating dissolution test profiles of immediate release tablets of ritonavir. The minimum data requirements to make useful predictions were assessed. ADMET predictor (part of DDDPlus) and Chemicalize (an online resource) were used to estimate pKa, logS and molecular charge. A surfactant model was developed to estimate the solubility enhancement in media containing surfactant. The software's transfer model based on the USP two-tiered dissolution test to mimic the in vivo transfer from stomach to small intestine was assessed. All simulations were compared with experimental results.

ADMET predictor without any real measurements showed lower drug solubility at pH 1.0 compared to data obtained from Chemicalize, which showed a higher solubility at pH 1.0. One measured data point was shown to be sufficient to make predictive simulations in DDDPlus. However, at pH 2.0 the software overestimated drug release while at pH 1.0 and 6.8 simulations were close to the measured values. A surfactant solubility model established with measured data gave good dissolution predictions. The transfer model uses a single vessel model and is at this point not suitable to predict the two in vivo environments separately because the composition of the two media in regard to their surfactant content cannot be differentiated.
For weak bases like ritonavir a minimum of three solubility data points is recommended for in silico predictions in buffered media. A surfactant solubility model is useful when predicting dissolution behaviour in surfactant media.

In the second study, solid dispersion of ritonavir was prepared through hot melt extrusion process. Dissolution test results of direct compressed tablets with and without disintegrant in various media with physiologically relevant pH were compared with simulations. Solubilizer and disintegrant effect were evaluated on the DDDPlus^{TM} simulation software using previously published solubility data on ritonavir. Observed and predicted dissolution profiles similarity tests and drug release mechanisms were assessed. Optimization of the Solubilizer Effect Coefficient (SEC) on the program give a good estimation of the effect of

copovidone in the extrudate on the dissolution profiles of all tablets. The SEC was dependent on the drug/polymer ratio and was therefore the same for both tablets with and without disintegrant. Disintegrant concentration in the program has no effect on simulations, rather the disintegration time was the main predictive factor. Drug release was formulation controlled in the tablets without disintegrant and in the tablets with disintegrant was via drug diffusion and polymer surface erosion.

In silico predictions need measured solubility data to be predictive. A combination of minimal experimental data and simulations can support the dissolution development at an early stage. DDDPlus™ has the potential to estimate the effect of excipients in a formulation on in vitro dissolution at an early stage in the drug development process. This could be useful in decisions on formulation strategies to enhance bioavailability in BCS class II and IV drugs.

Preface

This thesis is an original work by Juliet Obianuju Njoku, completed under the supervision of Prof. Raimar Löbenberg at the Drug Development and Innovation Centre (DDIC) at the University of Alberta.

Chapter 2 of this thesis has been published as Juliet Obianuju Njoku, Daniela Amaral Silva, Dwaipayan Mukherkjee, Gregory K Webster & Raimar Löbenberg with the title of "In silico tools at early stage of pharmaceutical development: data needs and software capabilities" in **AAPS PharmSciTech Journal** *June 2019, 20:243*.

Chapter 3 of this thesis is under review as Juliet Obianuju Njoku, Dwaipayan Mukherjee, Gregory K Webster & Raimar Löbenberg with the title "Amorphous solid dispersions in early stage of formulation development: predicting excipient influence on dissolution profiles using DDDPlus" in **Dissolution Technologies Journal**, 2019.

Dedication

To my parents Justine and Comfort Njoku for your unending love and support. From you I learnt to dream, to believe that where there is a will, there is a way.

To my dear husband, Patrick Onyechege for being there. Your delightful sense of humour encouraged and kept me going through it all.

To the light of my life, my son Jordan Onyechege. My world is complete and stable because of you.

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I appreciate the contribution of Dr Gregory Webster and Dr Dwaipayan Mukherjee from Abbvie Inc to my research, it was an incredible opportunity from which I learnt a lot. My appreciation also goes to Simulations Plus Inc for their

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The staff at the Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta deserve accolades for providing such a warm and welcoming environment for learning to students from all backgrounds and cultures.

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List of Abbreviations

ADMET	Absorption, Distribution, Metabolism, Elimination and Toxicity
API	Active pharmaceutical ingredient
BA	Bioavailability
BA/BE	Bioavailability / Bioequivalence
BCS	Biopharmaceutics classification system
CMC	Critical micelle concentration
DF	Dosage form
FDA	Food and Drug Administration
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
IR	Immediate release
i^{th}	Occuring at position *i* in a sequence
HIV	Human immunodeficiency virus
LogD	Distribution coefficient in logarithmic form
LogS	Solubility on a logarithmic scale
M	Molar
mg/mL	Milligram per milliliter
mL	Milliliter
mm	Millimeter

nm	Nanometer
pH	Measure of acidity or alkalinity on a logarithmic scale
pKa	Acid dissociation constant
PVP	Polyvinylpyrrolidone
rpm	Rotations per minute
R^2	Coefficient of determination
R^2adj	Adjusted coefficient of determination
SDS	Sodium dodecyl sulphate
SEC	Solubilizer effect coefficient
SEF	Solubility enhancement factor
QbD	Quality by design
USP	United States Pharmacopeia
µL	microliter
µm	Micrometer
%	Percent
°C	Degree centigrade

Chapter 1

Literature review

1.1 Introduction

A prospect that has shown increasing potential in reducing the amount of active pharmaceutical ingredient (API) necessary for drug product development is the use of mathematical models and simulation. Simulations are the application of mathematical models. In the pharmaceutical industry, mathematical-based models can be applied at all stages of the drug development process (1). The cost of developing a prescription drug is estimated at $2.6 billion, and it takes about 10 to 15 years from target selection to drug approval (2,3). Only about 35% of drug discovery candidates eventually qualify for clinical testing (4). There is a need for computational modeling methods with improved speed and performance to allow rapid in silico screening of drugs to increase success rates and reduce development time and cost. To facilitate the use of predictive modeling in formulation development, an in depth mechanistic understanding especially of poorly soluble molecules is required.

1.2 Drug solubility and bioavailability

The aqueous solubility of a drug plays an important role in the absorption of the drug after oral administration. The drug solubility influences the dissolution rate

at which the solid dosage form enters into solution that can be absorbed. Oral bioavailability depends on various factors which include aqueous solubility, dissolution rate, drug permeability, first-pass metabolism and susceptibility to efflux mechanisms (Figure 1.1) (5). The fundamental parameters influencing bioavailability of solid oral dosage forms are the aqueous solubility and drug permeability (6).

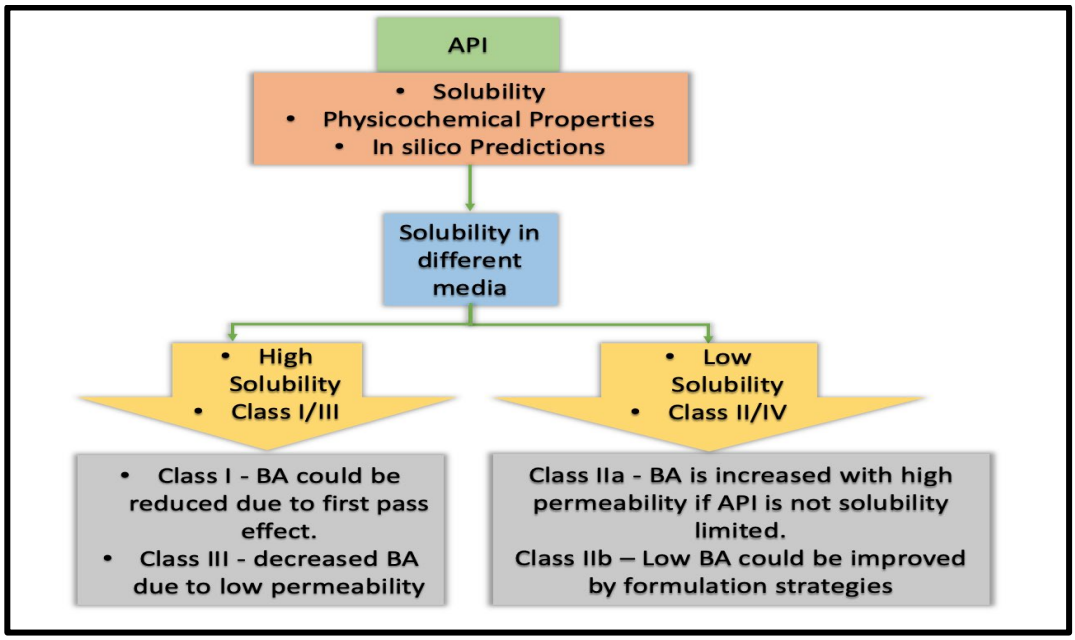


Figure 1.1 - Relationship between solubility of oral solid dosage forms and bioavailability (BA)

1.3 Drug solubilization mechanism in micelles

More than 40% of new drug candidates have low aqueous solubility. The poor and incomplete dissolution of these drugs limit their bioavailability and consequently, different approaches of improving solubility have been explored such as the solubilization of drugs in surfactant micelles (7). Surfactants are amphiphilic molecules composed of a hydrophilic or polar head and a hydrophobic or nonpolar tail. The surfactant head could be charged (cationic or anionic), dipolar (zwitterionic) or non-charged (nonionic) (7). Surfactants are utilized in a variety of drug dosage forms to improve wetting, stability and bioavailability of drugs (9). Above the critical micelle concentration (CMC), surfactant molecules form aggregates called micelles (10). The hydrophobic tails of the surfactant assemble in the interior of the micelle to limit their contact with water leaving the hydrophilic heads on the outside in contact with water (Figure 1.2) (11).

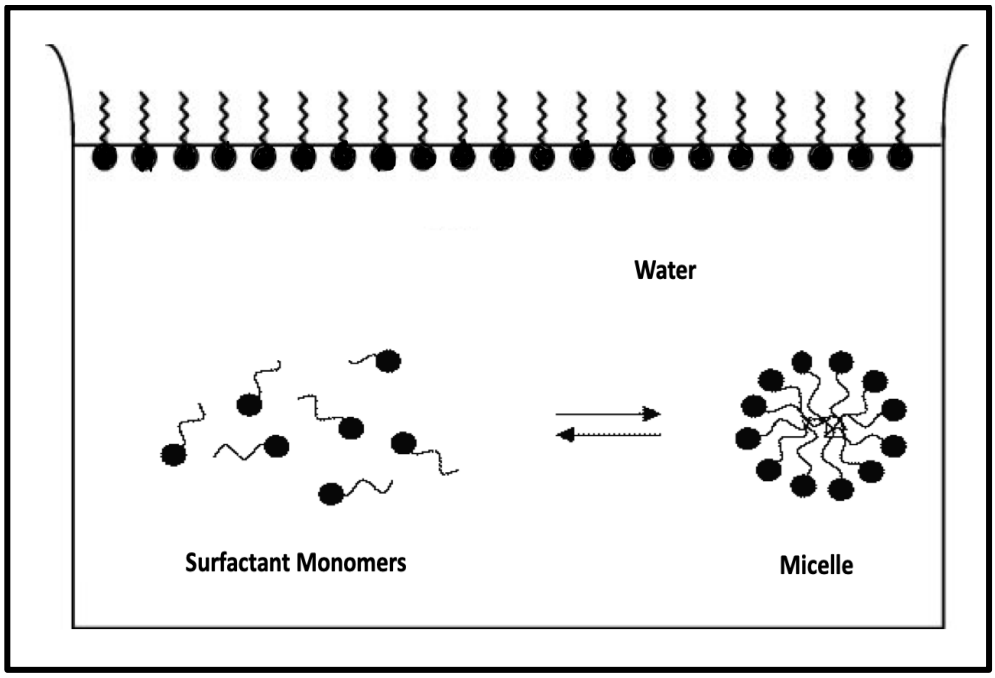
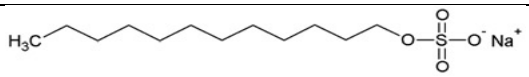
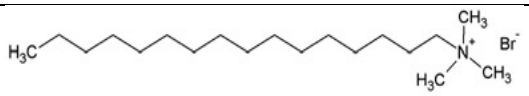
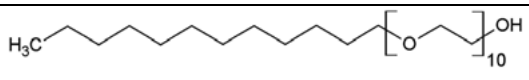
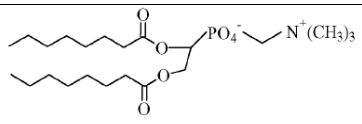


Figure 1.2 - An illustration of the aggregation of surfactant monomers to form micelles in a thermodynamic equilibrium. Adapted from ref (7).

Table 1.1 - Surfactants that are commonly utilized in dissolution testing

Trade name	Acronym used in texts	Molecular mass (g/mol)	Charge	Chemical structure
Sodium lauryl sulphate	$C_{12}SO_4Na$	288	Anionic	
Cetyl trimethyl ammonium bromide	$C_{16}TAB$	364	Cationic	
Polyoxyethylene (10) lauryl ether	$C_{12}E_{10}$	627	Nonionic	
1,2-Dioctanoyl-sn-Glycero-3-Phosphocholine	diC_8PC	509.6	Zwitterionic	

As micelles are formed by noncovalent aggregation of individual surfactant monomers, they are labile and their shape and size can vary based on solution conditions such as temperature, surfactant concentration and composition, ionic strength and pH (7). The Krafft temperature for micelle formation of SDS in water is about 15 ℃ (8).

Solubilization can be defined as an increase in the apparent aqueous solubility of the drug due to reversible interaction with the micelles of a surfactant in water to form thermodynamically stable solutions (7,12). The solubility of the drug remains low until the concentration of the surfactant reaches the critical micelle concentration (CMC). The drug solubilization efficiency of surfactant micelles can be assessed by the molar solubilization capacity (Equation 1.1), where *x* is a measure of the ability of the surfactant to solubilize the drug (7):

$$x = \left(\frac{S_{tot} - S_w}{C_s - CMC}\right) \text{ x } 1000$$

Equation 1.1

where *x* is the number of moles of the drug solute that can be solubilized by one mole of micellar surfactant, S_{tot} is the measured molar drug solubility in the

presence of surfactants, S_W is the intrinsic water solubility of the drug, C_S is the molar surfactant concentration, and CMC is the critical micelle concentration of the surfactant (7).

Studies by Wiedmann et al (2002) on the solubilization of drugs in bile salt micelles suggest that prediction of solubilization in the intestine is possible with in vitro measurements and adequate information on the appropriate micellar solutions. The FDA recommends that excipients such as surfactants to be used in dissolution testing should be used in quantities not in excess that can impact drug absorption but enough to fulfill its function and achieve clinical relevance (16). The physicochemical properties of a surfactant, the ionic strength and the nature of the buffer system all depends on the type of drug being studied (17). Therefore, the surfactant to be used should expedite the drug dissolution and enhance in vivo predictability. Sodium lauryl sulphate (2%w/v) has been shown to increase the solubility of fenofibrate (a poorly soluble BCS class II drug) by 2000 times as compared with its solubility in an aqueous phosphate buffer solution (18). The solubility of mefenamic acid is affected by a change in ionic strength when sodium lauryl sulphate is used, while cetyltrimethylammonium bromide (CTAB) does not show such effect (19).

1.4 Dissolution media recommendation for in vitro dissolution testing

The choice of a dissolution medium is an important factor in the dissolution of poorly soluble drugs because dissolution is the rate limiting step to absorption. The composition, volume and hydrodynamics of the contents of the lumen in vivo has to be adequately reproduced in vitro to predict limitations in dissolution of poorly soluble drugs (20). Dissolution depends on aspects such as pH, surfactant, buffer capacity and medium volume, therefore, the in vitro dissolution medium has to closely reflect these conditions as it is in the gastrointestinal tract (21,22). National pharmacopoeias recommend dissolution test media such as Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) to cover the physiological pH range of 1.2 to 7.5. The physiological pH range in the gastrointestinal tract under fasting conditions varies from 1.4 to 2.1 in the stomach, 4.9 to 6.4 in the duodenum, 4.4 to 6.6 in the jejenum and 6.5 to 7.4 in the ileum (23). However, for drugs which are not soluble at this pH range, surfactants can be incorporated to improve solubility (24). Dissolution testing with biorelevant media which are designed to mimic the complexity of human GI tract solutions may be useful for internal decision-making purposes during formulation development, however methods using biorelevant media are not necessarily biopredictive (linked to a compound's clinical behavior) unless such relationships have been established with clinical study data (25). The use of biorelevant media is cost-intensive and complex and for this reason simple buffer

systems are preferred for routine dissolution analysis. Typical dissolution media listed in the United States Pharmacopeia (USP) include dilute hydrochloric acid and buffers in the physiologic pH range of 1.2–7.5 (26,27). The type of medium and the volume selected should provide sink conditions. Sink conditions are described in the USP as the volume of the media being at least three times of that required to form a saturated solution of the drug substance (28). Sink conditions ensure that the amount of drug already dissolved in the media does not affect the dissolution rate as the experiment progresses. If sink conditions are not met, the dissolution rate will artificially slow down as the active pharmaceutical ingredient (API) nears the saturated solution state, making the dissolution test not reflective of in-vivo environment (28). Aqueous media in a pH range of low solubility should be buffered as SDS in concentrations lower than 0.23% act more like a wetting agent than as a solubilizing agent because this concentration is below its critical micelle concentration (CMC) (25). Dissolution medium volume commonly used in industry and accepted by regulatory agencies are 500ml and 900ml (25).

1.5 Dissolution testing as a quality control method

Dissolution testing is an important tool for evaluating the performance of oral solid dosage forms (28). In 1897, Noyes and Whitney conducted the first dissolution experiments and published an article titled “the rate of solution of

solid substances in their own solutions" (29). Since then, it has been used for decades to aid in formulation development and to ensure batch-to-batch quality, consistency and performance of drug products (25). L.J Edwards in 1951 appreciated that following the oral administration of solid dosage forms, if the absorption of the drug from the gastrointestinal tract is rapid, then dissolution is the rate-limiting step, thus linking drug dissolution with its bioavailability (30,31). For immediate-release solid oral dosage forms, USP Apparatus 1 (Basket) or Apparatus 2 (paddle) (Figure 1.3) are typically used in dissolution testing. Other dissolution testing techniques used for solid dosage forms include the USP Apparatus 3 (reciprocating cylinders), USP Apparatus 4 (flow-through-cell), USP Apparatus 5 (paddle-over-disk), USP Apparatus 6 (cylinder), USP Apparatus 7 (reciprocating holders) (26,32-34). With the paddle apparatus, a 50-rpm spindle speed is recommended as a starting point based on regulatory guidances from FDA (34), the European Medicines Agency (EMA) (36), and the Japanese Pharmaceutical and Food Safety Bureau (PFSB) (37). If there are issues with coning (the piling of non-dissolving excipients under the paddle that limits media penetration into the pile), the use of paddles with a 75-rpm spindle speed is recommended (25). Sampling time points are based on a drug's dissolution profile and usually in the range of 5 minutes to 60 minutes, the intervals between time points is also determined based on the drug's profile.

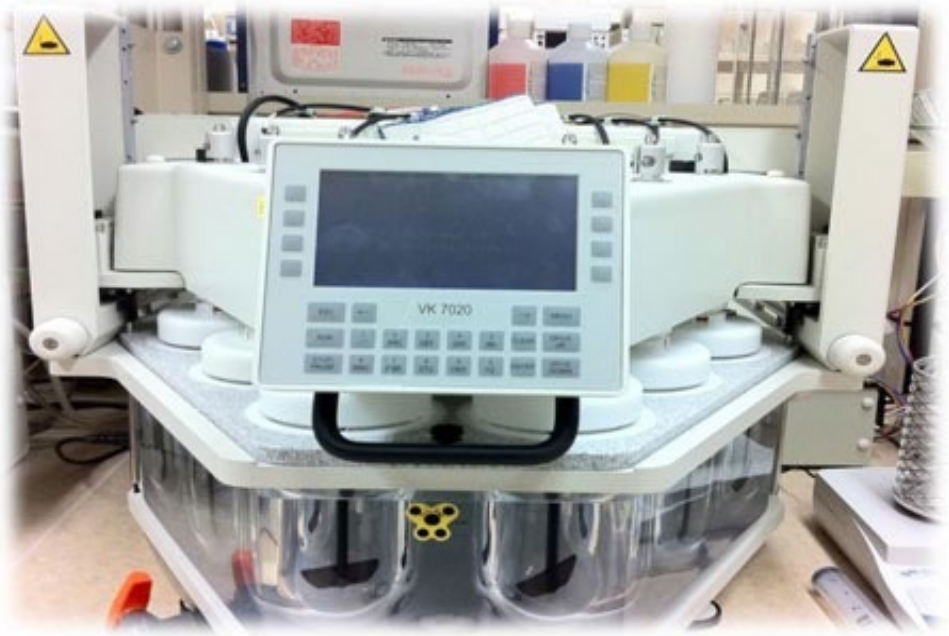


Figure 1.3 - The USP Dissolution Apparatus 2

The solubility versus pH profile can provide an insight during dissolution medium selection for initial examination of a compound (38). Surfactants should be incorporated if the medium in the pH range does not give sufficient dissolution, sodium lauryl sulphate (SDS) is the most common surfactant used, usually in the range of 0.1-3% (39).

1.6. The Biopharmaceutics Classification System (BCS)

The Biopharmaceutics Classification System (BCS) is a scientific framework for classifying a drug substance based on its aqueous solubility and intestinal permeability (40) into four classes as shown in Figure 1.4.

Figure 1.4 - The Biopharmaceutics Classification System

A drug is classified as highly soluble when its highest marketed dose strength is soluble in 250 ml of aqueous media over a pH range of 1–6.8 at 37 ± 1 °C (39,41). It is classified as highly permeable when the extent of absorption in humans is determined to be greater or equal to 85% of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose (39,41). The original BCS in the FDA Guidance for Industry (2000) waiver of in vivo bioavailability and bioequivalence studies for immediate release solid

oral dosage forms defines a highly soluble drug as one whose highest dose strength is soluble in ≤ 250ml of aqueous media over a pH range of 1 – 7.5, while a highly permeable drug is defined as a drug with an absolute bioavailability of 90% or more. The current pH range also aligns with the dissolution pH ranges of pH 1.0, 4.5 and 6.8 buffers.

1.6.1 FDA guidance for dissolution Testing of Immediate Release Solid Oral Dosage Forms

To determine drug solubility class, FDA recommends that "the pH-solubility profile of the test drug substance should be determined at 37 ± 1°C in aqueous media with a pH in the range of 1 - 6.8. A sufficient number of pH conditions should be evaluated to accurately define the pH-solubility profile within the pH range of 1 - 6.8. The number of pH conditions for a solubility determination can be based on the ionization characteristics of the test drug substance to include pH = pKa, pH = pKa + 1, pH = pKa - 1, and at pH = 1 and 6.8. A sufficient number of pH conditions should be determined for both ionizable and non-ionizable compounds. A minimum of three replicate determinations of solubility in each pH condition is recommended." (43). The bioavailability of a BCS class I and in some cases class III drug is not limited by dissolution if 85% of the drug is dissolved in 0.1N HCl in 15 minutes (39). The dissolution testing conditions

should be based on physicochemical characteristics of the drug substance and the environmental conditions the dosage form might be exposed to after oral administration. “Dissolution testing should be carried out using USP Apparatus 1 at 100 rpm or USP Apparatus 2 (typically at 50 rpm, or at 75 rpm when appropriately justified) using 500 mL (or 900 mL with appropriate justification) of the following dissolution media: (1) 0.1 N HCl or Simulated Gastric Fluid USP without enzymes; (2) a pH 4.5 buffer; and (3) a pH 6.8 buffer or Simulated Intestinal Fluid USP without enzymes..” (43). The use of surfactants such as sodium lauryl sulphate is encouraged for water insoluble or sparingly soluble drugs. An immediate release oral solid dosage form may be considered very rapidly dissolving if 85 percent or more of the drug substance dissolves within 15 minutes (43).

1.7 Solubility enhancement using amorphous solid dispersion

Formulations of solid dispersions have gained enormous attention as one of the many ways to improve solubility and consequently, bioavailability (44). Amorphous solid dispersions are based on hydrophilic polymers that dissolve in dissolution medium rapidly to enhance the dissolution rate of the formulation. Solid dispersion can be defined as ‘dispersion of one or more API in an inert carrier which is usually polymeric and amorphous in the solid state, prepared by either melting, solvent or the combined melting-solvent method’ (45,46). Hot-

Melt Extrusion is an established process for the manufacturing of solid dispersions which has been shown to improve wettability, flow properties and drug dissolution (47). Hot-melt extrusion has gained more recognition as the amount of poorly soluble chemical entities in drug development is rapidly increasing.

1.8 In silico prediction in early drug development

A predictive model is built as a representation of an underlying physical-chemical phenomenon (1). The first example of aqueous solubility prediction using computational methods was when Fühner in 1924 observed that the solubility of homologous series decreased with the addition of methylene groups (48). Solubility was estimated from a drug's physicochemical properties using quantitative structure-activity relationships. The prediction of aqueous solubility has slowly taken shape over the past 80 years. Molecular size is the most dominant indicator of solubility because aqueous solubility is controlled by interactions between water and the surface of a molecule (48,49). Other properties influencing solubility include hydrogen bonding, melting point, various atom and group contribution and molecular connectivities (48). Predictive models have become more complex as more properties that influence solubility have become more apparent. The application of in silico models has many advantages which include reduction of experimentation cost, improvement of productivity, and

comprehensive process understanding, it provides assistance in formulating new drug candidates by simplifying the selection and identification of new leads. When fewer experimental tests are needed for simulations, it makes its application even more beneficial by sparing the limited API available at the early stage of drug development. In silico models can replace in vitro tests under the right conditions.

DDDPlus™ (Dose Disintegration and Dissolution) software (version 5.0) used in this study, by Simulations Plus, Inc. (Lancaster, CA, USA) is one of such predictive in silico models used to simulate the dissolution behavior of different formulations by defining excipients and test conditions (50). The software is divided into three main tabs – Formulation, Dissolution Method and Simulation as described in Chapter 2.

Formulation tab

The API physicochemical characteristics and formulation parameters are defined in the formulation tab.

The formulation tab includes eight different dosage forms (DF) that the user can select: Immediate Release (IR) (tablet, powder, capsule, bead-coating), Controlled Release (CR) (polymer matrix, swellable polymer matrix) Bilayer Tablet and Delayed Release coated tablet. When tablet is selected as the dosage form one can also define its manufacturing properties, such as compression force, tablet diameter and disintegration time (14).

The formulation composition can be set up for excipients in the included database or self-defined ingredients can be added. The function of each ingredient in the formulation (API, disintegrant, polymer, etc), as well as the dissolution model (e.g mass transfer, Nernst-Brunner, intrinsic dissolution) can be defined by the user (14).

As stated by the DDDPlus user manual (14), the mass transfer dissolution model used in this study is based on the approach that dissolution of the solid is influenced by agitation of the solvent, the particle is assumed to be in a well-stirred solution surrounded by a boundary interface layer, and the rate of mass transferred from the interface layer into the solution is a product of the interfacial area, concentration difference and the mass transfer coefficient (Equation 1.2).

$$\frac{dMU}{dt} = -kA(\mathrm{C}s - \mathrm{C}b)$$

Equation 1.2

Where Mu is the amount of undissolved drug, A is the surface area (cm^2), C_s is the solubility at particle's surface, C_b is the bulk concentration and k is the mass transfer coefficient (cm/min) (14).

This model takes into consideration the hydrodynamics of the system unlike the Nernst-Brunner, Johnson-Spherical and Johnson-Cylindrical dissolution models which are based on a diffusion layer model which is independent of the velocity of the apparatus and fluid. The mass transfer coefficient for the mass transfer model is obtained from the medium viscosity and fluid velocity (14).

An excipient-specific coefficient, which represents the influence of the excipient on the formulation, and a calibration coefficient can be optimized using the Optimization module present in the software to better fit the observed data (14). An API's physicochemical properties (e.g, solubility, pka, diffusion coefficient, logP) can initially be predicted from its chemical structure using ADMET Predictor™ (Absorption, Distribution, Metabolism, Elimination and Toxicity Predictor) (Simulations Plus, Lancaster, CA, USA) module in DDDPlus™.

The pKa-based solubility model which can use the experimental solubility data of the drug can be optimized using the "Fit Model" button in the pKa table window. Under the Simulation Tab the option "Use Internal pKa-based Solubility Model" for solubility calculation can be chosen for simulations to include the fitted data.

Dissolution method tab

The dissolution parameters can be defined according to the in vitro dissolution test conditions used. The medium volume and constituents, USP

dissolution apparatus and rotation speed can be selected. For the media with surfactant, the surfactant type and concentration can be entered in the program and a surfactant solubility model can be built to define the API solubility vs. surfactant concentration in the media. For simulations involving surfactants, the program has a critical micelle concentration (CMC), molecular weight and aggregation number associated with each surfactant.

Simulation tab

The option to use either pKa-based solubility or experimental solubility in simulations can be found in this tab, the length of the simulation run time can be entered prior to running a simulation.

1.9 Ritonavir

Ritonavir is a protease inhibitor which is used in combination with other antiretroviral agents for the treatment of HIV-1 infection in adults and children of 2 years of age and older. It is administered at a dose of 100mg – 200mg twice daily and improves the bioavailability and half-life of other protease inhibitors (51). Ritonavir is the API of Abbott's antiretroviral drug Norvir, marketed as an oral liquid and semisolid capsules. Norvir; formerly ABT-538 was approved in 1996 as the second HIV protease inhibitor at a dose of 600 mg twice daily on the basis of demonstrated survival benefit; however, the drug is now used exclusively

as a pharmacokinetic booster at lower doses (100 mg once or twice daily) (52). Previous studies by Xu et al found ritonavir to have a solubility of 400 µg/mL in 0.1N HCl (pH 1) and 1 µg/mL at pH 6.8, 37 ℃ (53).

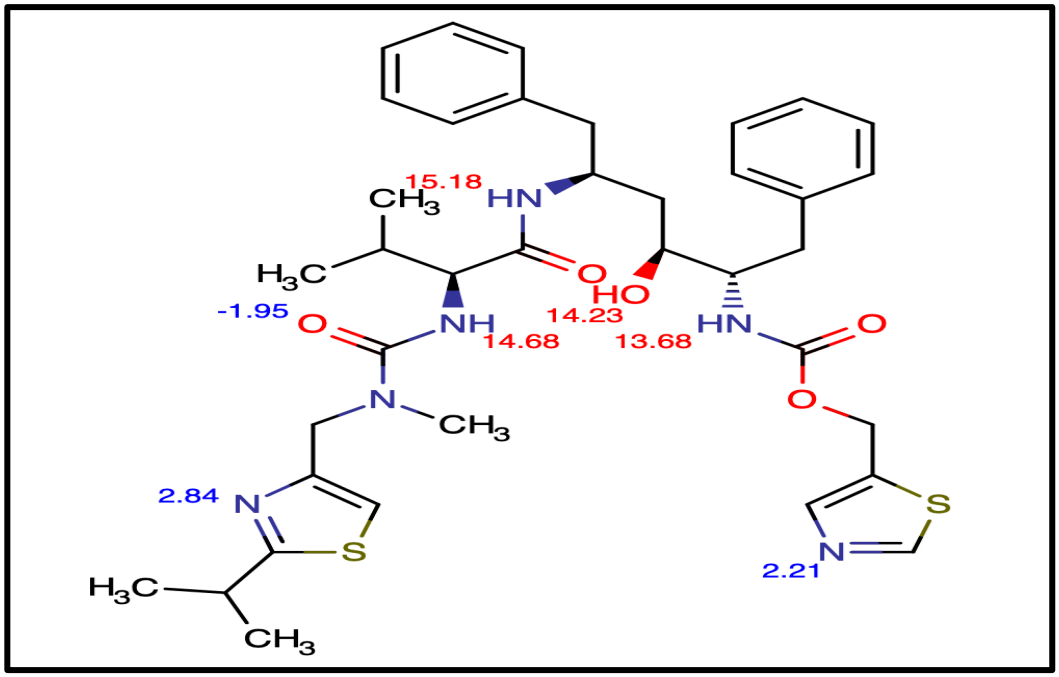


Figure 1.5 - Chemical structure of ritonavir - $C_{37}H_{48}N_6O_5S_2$ (molecular weight: 720.946 g/mol) showing the acidic pKa values in red and basic pKa values in blue.

The ionization pattern of ritonavir is influenced by its amphoteric nature (possessing both acidic and basic moieties). Its strongest acidic pKa is 13.68 while its strongest basic pKa is 2.84 (Figure 1.5).

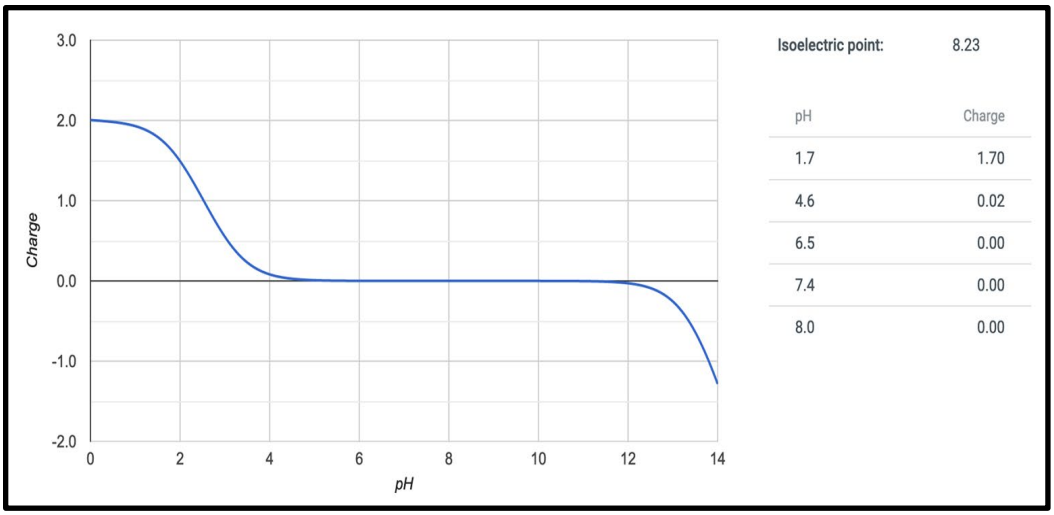


Figure 1.6 - Illustration of ritonavir's charge distribution in coloumb (C) across pH values. The isoelectric point is the pH at which ritonavir has no electric charge and is neutral.

Ritonavir exhibits a pH-dependent solubility and a complex solubility pattern due to the pH gradient in the gastrointestinal tract. In its ionized form it dissolves in the acidic pH of the stomach, as it moves along to the small intestine where the pH is higher it may precipitate.

1.10. Hypothesis

- The DDDPlus^{TM} software program has potential benefits in saving costs and reducing time spent in early drug development.

The work hypothesis for the first study:

- The solubility of a drug in a medium will be sufficient to predict the dissolution profile of that drug in different media.

The work hypothesis for the second study:

- Computer simulations can be used to predict the effect of formulation strategy such as solid dispersion on the dissolution rate of a poorly soluble drug.

1.11 Objectives

The main purpose for this research was to test the hypotheses above through a mechanistic study of the various physicochemical parameters required to improve prediction accuracy in simulation for immediate release tablets in early drug development using the following methods:

i. Comparison of data obtained from different physicochemical property predictive platforms – the ADMET predictor (from Simulations Plus Inc. and available within DDDPlus^{TM}) and the Chemicalize database for their abilities to create a suitable solubility vs pH profile that can be used to make simulations of in vitro dissolution tests.

ii. Determination of number of data points of solubility as a function of pH that would be adequate for simulations of in vitro dissolution test.

iii. Exploring solubility optimization models in software program when using surfactant and comparing with experimental results.

iv. Evaluation of a two-tiered dissolution model to mimic drug transfer along the gastrointestinal tract.

v. Assessment of drug release mechanisms of different formulations using drug release models in a software program.

vi. Evaluation of solubilizer and disintegrant effect on the dissolution profile of ritonavir, a poorly soluble drug.

Chapter 2

In silico tools at early stage of pharmaceutical development: data needs and software capabilities

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2.1 Abstract

In early drug development, the selection of a formulation platform and decisions on formulation strategies have to be made within a short timeframe and often with minimal use of the active pharmaceutical ingredient (API). At this stage, there is limited information available about the physicochemical and biopharmaceutical properties of a new drug candidate. The current work evaluated the various physicochemical parameters required to improve the prediction accuracy of in silico tools on the dissolution profiles of immediate release tablets in early drug development.

DDDPlus^{TM} (Dose Disintegration and Dissolution Plus) was used in simulating dissolution test profiles of immediate release tablets of ritonavir. The minimum data requirements to make useful predictions were assessed. ADMET predictor (part of DDDPlus) and Chemicalize (an online resource) (52) were used to estimate pKa, logS and molecular charge. A surfactant model was developed to estimate the solubility enhancement in media containing surfactant. The software's transfer model based on the USP two-tiered dissolution test to mimic the in vivo transfer from stomach to small intestine was assessed. All simulations were compared with experimental results.

ADMET predictor without any real measurements showed lower drug solubility at pH 1.0 compared to data obtained from Chemicalize, which showed a higher solubility at pH 1.0. One measured data point was shown to be sufficient to make

predictive simulations in DDDPlus. However, at pH 2.0 the software overestimated drug release while at pH 1.0 and 6.8 simulations were close to the measured values. A surfactant solubility model established with measured data gave good dissolution predictions. The transfer model uses a single vessel model and is at this point not suitable to predict the two in vivo environments separately because the composition of the two media in regard to their surfactant content cannot be differentiated.

For weak bases like ritonavir a minimum of three solubility data points is recommended for in silico predictions in buffered media. A surfactant solubility model is useful when predicting dissolution behaviour in surfactant media. In silico predictions need measured solubility data to be predictive. A combination of minimal experimental data and simulations can support the dissolution development at an early stage. Further studies are needed to include excipient effects.

2.2 Introduction

Ritonavir is a lipophilic drug with a LogP of 4.2 (ADMET Predictor) and a weak base with pKa values of 2.84 and 13.68 (52). Systematic studies by Law et al (2001) (53) show that Ritonavir has a LogD of 4.3 at 25° C at pH 6.8. It is poorly soluble at a high pH (400µg/mL in 0.1N HCl, 1µg/mL at pH 6.8, 37° C), and has a slow dissolution rate (0.03mg/cm^2-min in 0.1N HCl at 37° C) (51).

Compounds with low aqueous solubility often suffer from limited bioavailability. If a low solubility drug candidate has reasonable membrane permeability, then often the rate-limiting process in absorption is the dissolution of the drug dose in the gastrointestinal tract (54,6). This is often the case for poorly soluble drugs (56). It is estimated that up to 40% of drug candidates have been abandoned due to insufficient solubility and associated poor pharmacokinetics under physiological conditions (55). Hence, approaches such as the use of in silico simulations based upon the drug's physicochemical properties promise an option to accelerate the selection between drug candidates, with less intensive in vitro testing. Solubility screening of compounds can reduce considerably the time and effort required to identify a lead compound (46).

A fundamental understanding of the physicochemical properties such as logD, solubility and excipient effects are imperative to develop a formulation strategy. In vitro dissolution characteristics must be thoroughly assessed, each step of the in vitro dissolution process must be studied under a variety of physiologically relevant conditions and multiple pH values need to be tested (56). The bioavailability (BA) of an API depends on the physicochemical properties and the key BCS parameters, solubility and permeability (24). The prediction of the in vivo dissolution behavior is therefore a key to estimate BA.

DDDPlus™ (Dose Disintegration and Dissolution Plus designed by Simulations Plus Inc., is a commercially available computer program used to simulate in vitro dissolution tests. USP apparatuses 1 (basket), 2 (paddle), 4 (flow-through cell) and rotating disk (intrinsic dissolution) methods are embedded on its platform (57). Previous studies by Duque et al (2017) and Almukainzi et al (2015) have reported its use to simulate dissolution of poorly water-soluble drugs (57,58). Uebbing et al (2017) utilized the software to justify the substitution of dissolution with disintegration testing as the quality control method for immediate release oral dosage forms. Abend et al (2019) demonstrated that the software had good predictability of dissolution performance in surfactant-containing media which can be useful during dissolution method development.

However, little information is available about the relevant physicochemical parameters that are required to obtain useful simulations in early drug development. Furthermore, there is no universal method described on how to obtain such data. The primary aim of this study was to outline the simulation process by creating a guideline that describes the required parameters, compare data obtained from different physicochemical property predictive platforms – the ADMET predictor (from Simulations Plus Inc. and available within DDDPlus™) and for example Chemicalize database for their abilities to create a suitable solubility vs pH profile that can be used to make simulations of in vitro dissolution tests.

2.3 Materials and Methods

2.3.1 Materials

Ritonavir powder was provided by AbbVie Inc (Chicago, IL, USA). Microcrystalline cellulose (Avicel® PH-102 NF) was obtained from FMC Biopolymer (Philadelphia, PA, USA), croscarmellose sodium was purchased from PCCA Canada (London, ON, Canada), magnesium stearate from H.L. Blachford Ltd (Mississauga, ON, Canada), hydrochloric acid (P.A 36.5%) was purchased from Fisher Scientific (Fair Lawn, NJ, USA), and sodium dodecyl sulphate was purchased from Caledon Laboratories Ltd (Georgetown, ON, Canada). HPLC grade water and water for the dissolution test media were generated in an Elgastat Maxima UF and an Elgastat Option 3B water purifier by ELGA Laboratories Ltd. (Mississauga, ON, Canada) and filtered through a Durapore® 0.22 μm GV filter by Millipore Canada Ltd. (Etobicoke, ON, Canada; for HPLC mobile phase). Acetonitrile HPLC grade was purchased from VWR International LLC. (Radnor, PA, USA) and filtered through a Durapore® 0.45 μm HV filter by Millipore Canada Ltd. (Etobicoke, ON, Canada).

2.3.2 Methods

Dissolution media (hydrochloric acid 0.1M, hydrochloric acid 0.01M and phosphate buffer pH 6.8) were prepared according to USP specifications (14), these

media were chosen to assess pH effect. Phosphate buffer (pH 6.8) was also used with three different sodium dodecyl sulphate (SDS) concentrations of 0.1%, 0.25% and 0.5% to assess surfactant effect. Immediate release tablets were prepared by direct compression at one metric ton pressure for 30 seconds using a Carver Laboratory Press by Fred S. Carver Inc. Hydraulic Equipment (Manomonee Falls, WI, USA). The final formulation composition is described in Table 2.1.

Table 2.1 – Ritonavir immediate release tablet formulation

Ingredient	**Amount (mg)**
Ritonavir (API)	10, 100
Microcrystalline Cellulose (Avicel ph-102 NF)	743
Croscarmellose Sodium	24
Magnesium Stearate	8

2.3.2.1 Solubility and Dissolution Testing

The solubilities of ritonavir in eight different media, were determined using the equilibrium solubility test (Shake flask method) (17). 5ml of different media (HCl 0.1M, HCl 0.01M, HCl 0.001M, HCl 0.0001M, Phosphate buffer 6.8 and Phosphate buffer 6.8 with 0.1%, 0.25%, 0.5% SDS) were saturated with ritonavir drug powder. The vials were shaken for 72 hours at room temperature to assure equilibrium. Samples (1.0 mL) were collected without replacement at each time point (24, 48 and 72 hours) and centrifuged at 15,000 rpm in a Biofuge^{TM} centrifuge

by Heraeus Instruments Inc. (USA) for 15 minutes. The supernatant (500 µL) was used for the HPLC analysis. The pH of the solubility and dissolution media was measured using an Accumet® XL 20 pH-meter by Fisher Scientific (Fair Lawn, NJ, USA).

Dissolution testing was performed using a VK 7020 system from Varian Inc. (Cary, NC, USA) equipped with 70 µm Full Flow™ Filters (Varian Inc.) and a VK 8000 auto sampler (Varian Inc.). All tests were performed with USP Apparatus 2 at 75 rpm rotation speed, 37 °C and using 900 mL of six types of dissolution media (HCl 0.1M, HCl 0.01M, phosphate buffer 6.8 and phosphate buffer 6.8 with 0.1%, 0.25%, 0.5% SDS). The dissolution media were deaerated by filtration, ultrasound and vacuum. A dissolution profile with multiple time points in systems which include low pH and surfactants is required for slowly dissolving drugs like ritonavir, thus samples (1.0 ml) were collected by the autosampler at each time point (3, 5, 10, 15, 20, 30, 45, and 60 minutes) without replacement and analyzed via HPLC.

2.3.2.2 HPLC Analysis

A 2 mg/ml standard solution in acetonitrile and monobasic potassium phosphate (1:1) was used for HPLC quantification of ritonavir. The calibration curve range was from 3.75% to 120% of the expected maximum drug concentrations in the medium. A VP-class Shimadzu Scientific Instruments (Kyoto,

Japan) liquid chromatograph, equipped with a Lichrospher® 60 RP Select B column (5 µm, 12.5x4 mm, by Merck Darmstadt, Germany) with a matching guard column and connected to a CBM-20A system controller, two LC-10AS pumps, an SIL-10ADVP auto sampler and an SPD-M10AVP diode array detector, was used. The system was controlled using the data acquisition software "EZ Start 7.4" (Shimadzu). The mobile phase was deaerated before use, using a combination of vacuum filtration, and ultrasound. The isocratic mobile phase was composed of acetonitrile, water and trifluoracetic acid 57:43:0.1 (v/v/v) and the flow rate was 1 ml/min. An injection volume of 50 µL was used without dilution and the retention time for ritonavir was approximately four minutes with a total run time of eight minutes. A wavelength of 240 nm was selected for the analysis.

2.3.2.3 DDDPlus™ Simulation Software

Formulation tab

In this study the IR:Powder dosage form was selected, since not much is known about excipient effect on simulations at this stage. Only one ingredient (the API) was selected for this dosage form option. The mass-transfer dissolution model was used for dissolution profile predictions.

Dissolution method tab

The dissolution parameters were set according to the in vitro dissolution test conditions used. Briefly: 900 mL medium, USP apparatus 2 (paddle), 75 rpm rotation speed and medium type (HCl 0.1M, HCl 0.01M, phosphate buffer 6.8 or phosphate buffer 6.8 with 0.1%, 0.25%, 0.5% SDS).

For simulations involving surfactants, the program had an assigned critical micelle concentration (CMC), molecular weight and aggregation number which are 0.008M, 288.4g/mol and 55 respectively for sodium dodecyl sulphate. The solubility of ritonavir is related to the surfactant concentration through the equation:

$$C_s = C_s(pH)[1+k*(C_{sur} - CMC)]$$

Equation 2.1

where *Cs* is the solubility of ritonavir adjusted for the surfactant effect (units of mg/ml), *Cs(pH)* is ritonavir solubility in the bulk fluid at a particular pH (in mg/ml), *Csur* is the surfactant concentration (in M), *CMC* is the surfactant's critical micelle concentration (units of M), and *k* is an optimizable parameter defined as the solubility enhancement factor (in units of 1/M) (14).

The solubility enhancement factor (SEF) is an equilibrium parameter that must be calibrated to an experimental dataset to quantify the interaction between the surfactant's concentration and the API solubility (14). To use this tool, the surfactant solubility data for ritonavir had to be previously determined experimentally. From the surfactant solubility data, the SEF for ritonavir was calculated as described above. The optimized values were exported to the database and used for our simulations.

A further set of simulations including two-tiered dissolution was performed. The medium selected to perform this simulation was phosphate buffer 6.8 USP with surfactant to emulate the bile salts effect as the DF transits from the stomach (pH set to 2) to the intestine (pH set to 6.8). The medium pH was set to 2 for the first 20 minutes, and from then the pH was increased to 6.8. The medium composition did not change, because the pH, volume, rotation speed and time are the only parameters that can be changed for two-tiered dissolution model in the program. The solubility test results indicated that the highest dose of ritonavir (100mg) would dissolve in 250ml of phosphate buffer + 0.25% SDS, therefore this media was selected as the second phase to run the simulation.

Simulation tab

Single simulations were performed for each experiment using 60 minutes simulation length, according to the experimental design. Data input used in the

program for simulations are listed in Table 2.2. Simulations using both loaded solubility values and pKa-based solubility were performed and compared with each other. The simulated dissolution profiles were compared to the in vitro results. The option to "Use Internal pKa-based Solubility Model" for solubility calculation was chosen for the simulations.

Table 2.2 – Ritonavir's physicochemical properties data input in DDDPlus for simulation

Parameter	**Ritonavir**
Amount (mg)	10,100
Molecular Weight (g/mol)	720.96
Solubility (mg/ml)	0.57 at pH 1 0.01 at pH 2
pKa	2.84,13.68
LogP	4.2
Particle Density(g/mL)	1.2
Precipitation Time (s)	900
Diffusion Coefficient ($cm^2/s \times 10^{-5}$)	0.44

2.3.2.4 Statistical Methods

Observed and simulated dissolution profiles were compared using f2 statistics test for similarity. DDSolver, an excel add-in in Microsoft Excel^{TM} designed for dissolution profile data analysis such as profile comparison or modeling (61), was used in the evaluation. The coefficient of determination (R^2) for evaluating in silico

data fit to in vitro data was obtained from DDDPlus^{TM}. The Korsmeyer Peppas and Gompertz model was used to determine the drug release mechanism after model fitting in DDSolver. Drug release values less than 65% were chosen for the modeling and where values were above 65%, the three lowest values were used.

2.4 Results

Experimentally determined solubility (Table 2.3) shows the pH-dependent solubility of ritonavir and its increased solubility with higher surfactant concentrations. At low pH values the API was more soluble due to its ionization state and microspecies distribution at such pH values as shown in Table 2.3 and Figure 2.1 respectively. The pKa-based solubility model using the predicted pKa value from ADMET predictor^{TM} underestimated ritonavir solubility at a lower pH, whereas data obtained from Chemicalize, an online prediction resource was more accurate (Fig. 3A and B, respectively). The pKa-based solubility model built from ADMET predictor^{TM} derived pKa values remained unchanged even when experimental solubility input was varied across different pH values. Therefore, the pKa values of 13.68 and 2.84 obtained from Chemicalize were used in all simulations.

Table 2.3 – Solubility test result of ritonavir in different media with physiologically relevant pH values as recommended by the FDA Guidance for Industry (41)

Media	Solubility (mg/ml)
0.1M HCl (pH 1)	0.57
0.01M HCl (pH 2) 0.001M HCl (pH 3) 0.0001M HCl (pH 4)	0.01 0.007 0.005
Phosphate Buffer USP 6.8	0.002
Phosphate Buffer USP 6.8 + 0.1% SDS	0.223
Phosphate Buffer USP 6.8 + 0.25% SDS	0.431
Phosphate Buffer USP 6.8 + 0.5% SDS	0.889

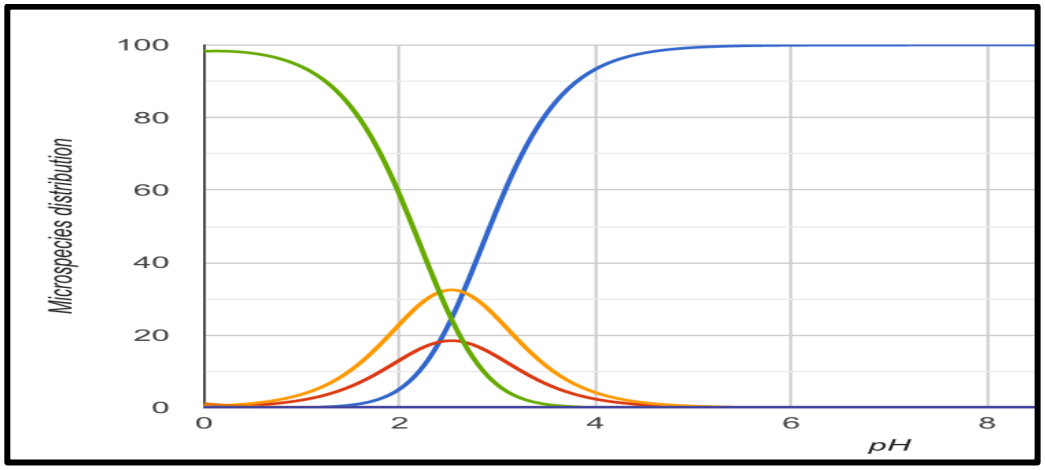


Figure 2.1 – Microspecies distribution of ritonavir functional groups obtained from Chemicalize database, the green line represents the microspecies distribution of the functional groups with its strongest basic pKa.

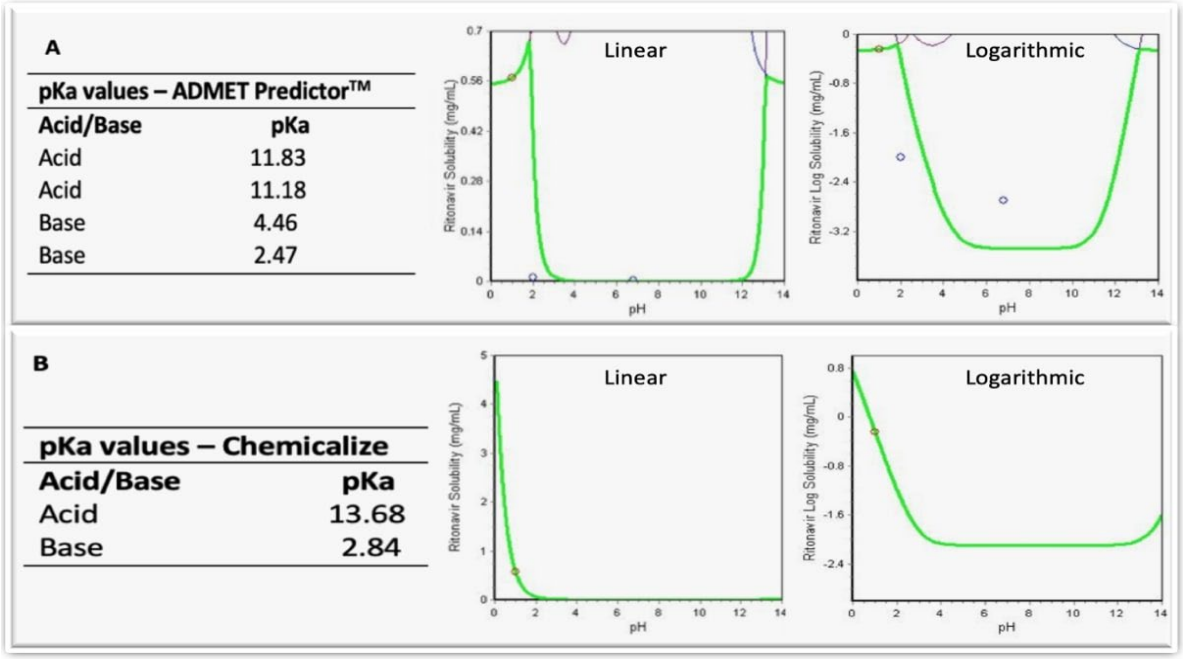


Figure 2.2 - Solubility vs pH profile using pKa values from ADMET predictor module (A) and pKa values from Chemicalize (B) presented in linear and logarithmic scales. The points in the plots represent measured solubility values. Plot B has one measured solubility value which indicates that one data point is sufficient to create a solubility vs PH profile for simulation

Three data points of experimental solubility measurements gave a profile that was sufficient to make simulations that will ensure accurate predictions throughout the physiological pH range.

The dissolution profiles of ritonavir in the various media are shown in Figure 2.3. The similarity factor (f2) between observed and predicted profiles is shown in Table 2.4. The predictions at pH 1.0 and 6.8 showed a high similarity to the observed values, while the prediction at pH 2.0 overestimated the drug release and was not

similar to observed values. When the reference pH and solubility (at reference pH) to run the simulations was set to pH 2.0 and 0.01 mg/ml, respectively (as measured), the predictions were found to be similar to the observed data.

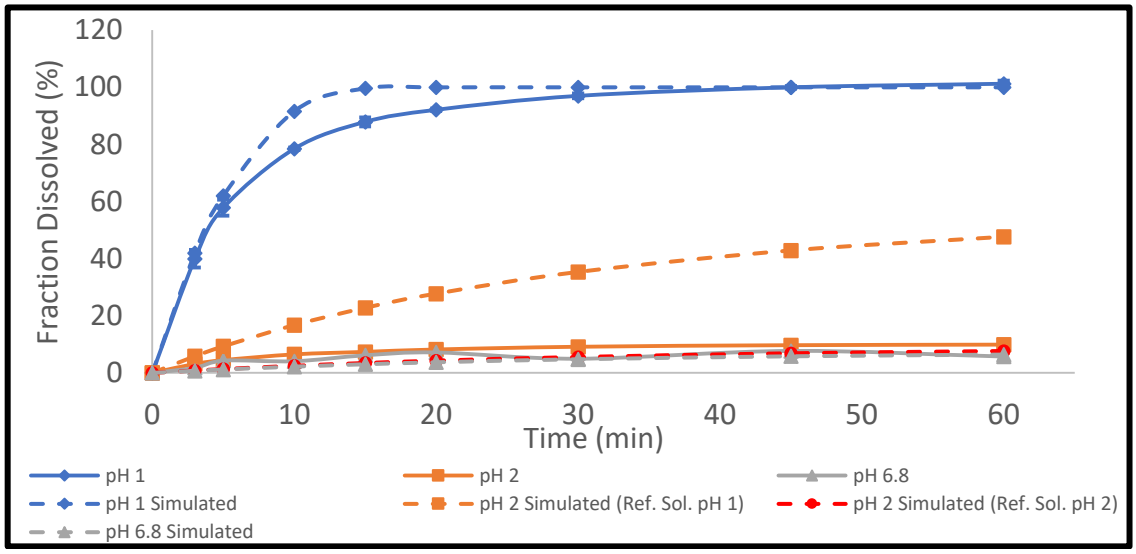


Figure 2.3 – Dissolution of ritonavir immediate release tablets (100 mg) in pH 1, pH 2 and pH 6.8 media and simulated profiles

Table 2.4– f2-test results comparing in silico to in vitro data, scores above 50 indicate similarity between compared profiles.

Compared Profiles	f2 Test (Accepted?)
Dissolution at pH 1.0	57 (yes)
Dissolution at pH 2.0	34 (no)
Dissolution at pH 2.0 (pH 2 solubility as reference solubility)	74 (yes)
Dissolution at pH 6.8	82 (yes)
Dissolution in phosphate buffer USP 6.8 + 0.1% SDS	66 (yes)
Dissolution in phosphate buffer USP 6.8 + 0.25% SDS	67 (yes)
Dissolution in phosphate buffer USP 6.8 + 0.5% SDS	69 (yes)

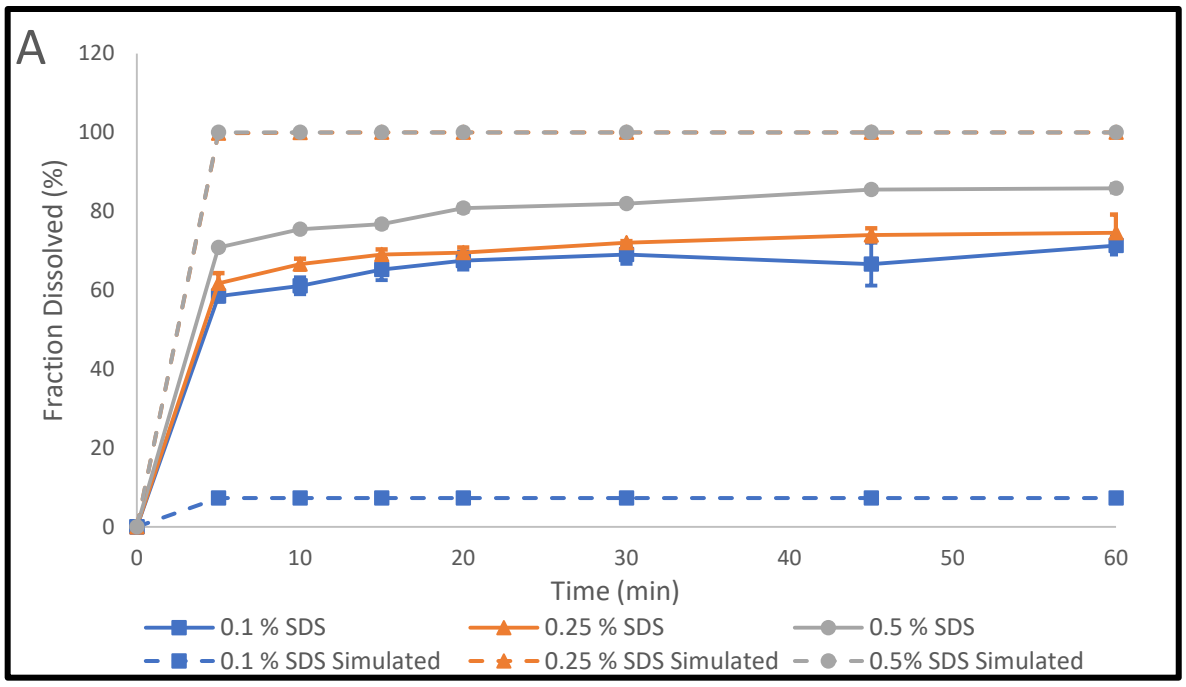
The observed dissolution profile for ritonavir 10 mg in phosphate buffer USP 6.8 with three different SDS concentrations was less than 100% release (Figure 2.4). Solubility test results of ritonavir in these media indicate that 100% drug release should be expected. This may be attributed to the lipophilic nature of ritonavir (Log P = 4.2) and its tendency to adhere to the vessel wall and paddle during the dissolution test, drug residues were observed when the apparatus was cleaned after the experiments. This may result in loss of material, as the tablet contained a low dose of 10 mg (62). In contrast, the in silico model predictions were based on the API's solubility as input and the selected drug dissolution model (mass transfer), hence it predicted 100% release without accounting for loss.

To circumvent this problem, immediate release tablets with 100 mg dose were made and tested under the same conditions as the previous formulation. As expected, the release was much higher, and as shown in Figure 2.4C, in media containing 0.25% SDS, 100% drug release was reached, which shows that an increase in the concentration of the drug will account for losses during dissolution testing due its lipophilicity and adsorption to surfaces.

Simulation of the dissolution profile of ritonavir in phosphate buffer USP 6.8 and SDS without building a surfactant model (using experimentally determined surfactant solubility) showed 100% of the drug dissolved in 15 mins in media containing 0.25% and 0.5% sodium dodecyl sulphate. This is because the concentration of the surfactant exceeds the CMC for SDS (0.008M), therefore the

program deduces that micelles will be formed, hence further solubilization will occur. This is not the case for the media containing 0.1% SDS, as the concentration of surfactant was well below its CMC. After fitting the data, the optimized surfactant solubility model was able to make suitable predictions for the 10 mg dose (Figure 2.4B).

There was an overprediction for the early time points, which can be attributed to disintegration time, which the software has not taken into account since IR: Powder was selected as DF and in early development excipient effects are not studied. Overall, simulations displayed acceptable f2 values (Table 2.4).



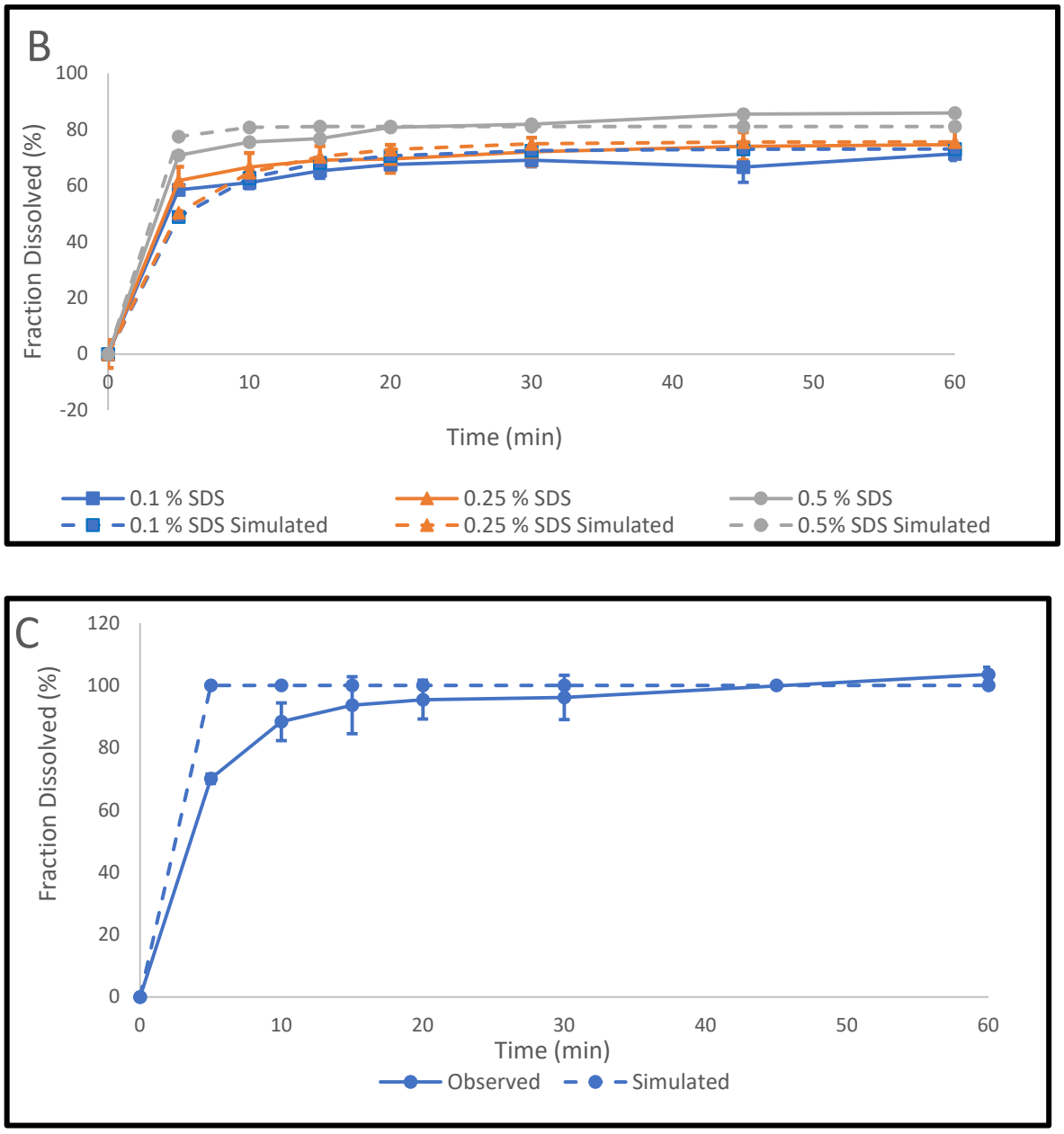


Figure 2.4 - Dissolution of Ritonavir 10mg IR tablets in phosphate buffer USP 6.8 and SDS - before optimization (A) and after optimization (B); dissolution of ritonavir 100mg IR tablets in phosphate buffer USP 6.8 and 0.25% SDS without optimization(C).

The Korsmeyer-Peppas model showed n values of 0.527, 0.581, and 0.455 for media with pH 1, 2 and 6.8. Media containing phosphate buffer 6.8 and 0.1%, 0.25%, 0.5% SDS had n values of 0.097, 0.103, 0.075 respectively. The immediate release tablets showed good fits ($R^2_{adj} > 0.8$) for the Gompertz model in all media except the media with pH 6.8 in which the tablets had very low solubility.

When using the two-tiered dissolution with pH change from 2.0 to 6.8, there was an overestimated prediction at pH 2 during the first 20 minutes at the acid stage. (Figure 2.5).

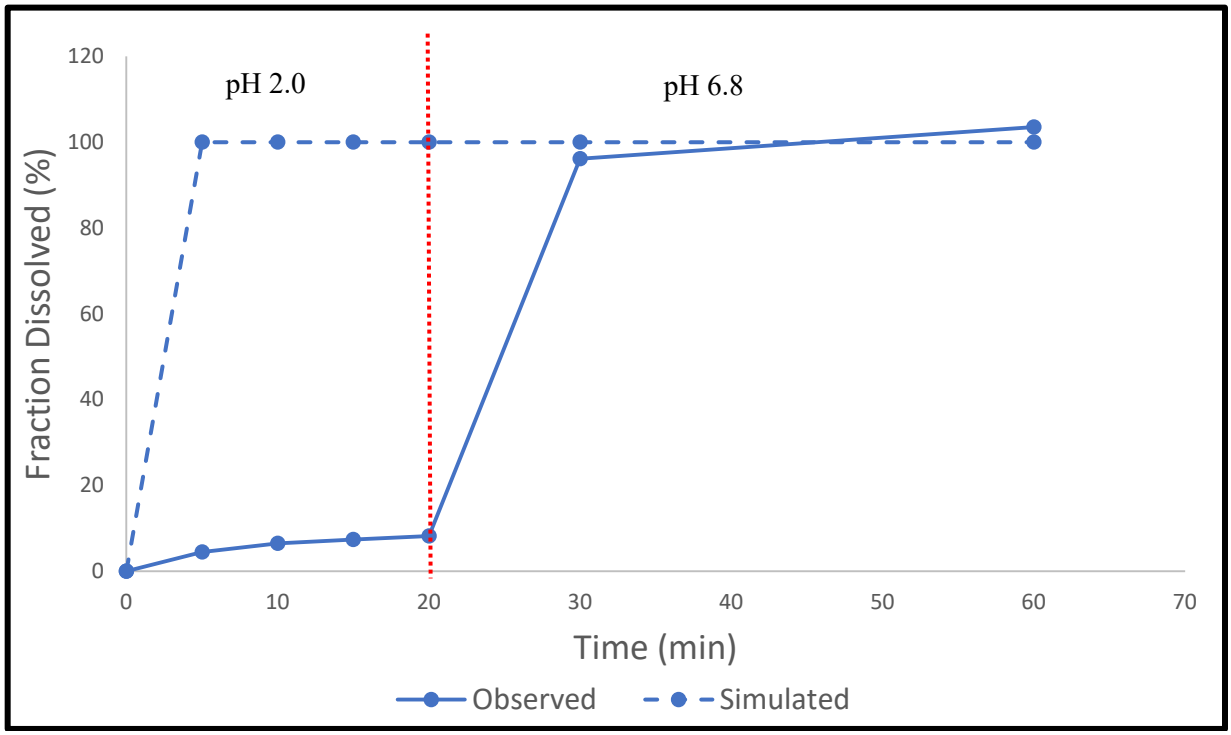


Figure 2.5 – Observed and Simulated two-tiered dissolution profile to simulate the passage of a drug from the stomach to the duodenum.

2.5 Discussion

Ritonavir is a weak base (strongest basic pKa at 2.84) with pH-dependent solubility and is a highly lipophilic drug (Log P 4.2) resulting in a low aqueous solubility at intestinal pH values (5-7.5). The solubility of a drug has implications on its in vivo performance and therefore is of utmost importance in early drug discovery (63). As demonstrated in this study, solubility estimation can be done based on the API's chemical structure and pKa values. For Ritonavir the values predicted by ADMET Predictor^{TM} showed poor predictive power, whereas the values retrieved from Chemicalize yielded more accurate predictions. However, this shows how different computer programs and databases can be used in combination at early development in a complementary way, uplifting the predictive power of in silico tools.

According to the FDA (41), "the pH-solubility profile of the test drug substance should be determined at 37 ± 1^{o}C in aqueous media with a pH in the range of 1 - 6.8. A sufficient number of pH conditions should be evaluated to accurately define the pH-solubility profile within the pH range of 1 - 6.8. The number of pH conditions for a solubility determination can be based on the ionization characteristics of the test drug substance to include pH = pKa, pH = pKa

+ 1, pH = pKa - 1, and at pH = 1 and 6.8." The maximum number of evaluated data points in this study was five, and it included the solubility at pH 1, 2, 3, 4 and 6.8.

DDDPlus^{TM} designs the solubility vs pH profile based on the pKa of the drug. Our evaluation of the minimum data points required to create a solubility vs pH profile showed little difference between profiles with one, two, three or four data points. However, three data points was chosen as the minimum recommendation to ensure accurate predictions throughout the physiological pH range during simulation of dissolution profiles.

Using an accurate solubility model is essential when predicting drug dissolution in different media based on parameters such as solubility, diffusion coefficient, diffusion layer thickness, bulk/micro-climate pH combined with few experimental tests (48).

In this study the dissolution of the formulation used (Table 2.1) was mostly API controlled as described by Uebbing et al., (48) i.e. dissolution depended only on the drug particle properties, with fast and complete disintegration. Thus, the excipient and formulation factors are not important at this stage of the formulation development. With this in view, it was justifiable to use IR:Powder as the dosage form model for the simulations.

For a weak base such as ritonavir with high solubility at pH 1, it is expected that 100% of the drug being dissolved in the stomach under normal pH conditions (about 1.2). Simulated profiles at pH 1 were in accord with this rationale, yet

DDDPlus™ predicted a faster dissolution rate than the observed data (Figure 2.3) which might be attributed to formulation effects in the early time points which were not part of this study. Nevertheless, both observed and simulated profiles showed above 85% of the drug dissolved in 15 mins. The suitable fit between observed and simulated profiles (R^2 = 0.88 and f2 test: 57) indicates that the program is capable of making suitable predictions at pH 1.

For drugs with pH- dependent solubility like ritonavir, the in silico model requires a pH reference solubility to match the dissolution test medium pH. As expected, when ritonavir's solubility at pH 2 (0.01mg/ml) was used as a reference solubility, the simulation had better correlation with the observed dissolution profile (Figure 2.3). This is a clear example of how simple experimental data enable computer simulations to reflect in vitro observations, which is a useful tool to reduce laboratory work and avoid trial and error experiments.

Surfactants are organic compounds with amphiphilic attributes due to hydrophilic groups head and hydrophobic groups tail in the surfactant monomer. An increase in the concentration of surfactants causes the formation of micelles, a self-association of multiple surfactant molecules creating a new colloidal phase of a hydrophobic core of surfactant tail (63). The concentration at which this phase change occurs is called the critical micelle concentration (CMC) (63). Hence, surfactants reduce the surface tension in the media thereby aiding material wetting and solubilization (64). Solubilization agents such as SDS can be used as

thermodynamic inhibitors to increase saturation solubility and subsequently reduce the degree of supersaturation (665). The gastrointestinal tract has bile acids and natural surfactants, however synthetic surfactants are being used in dissolution media instead of bile salts for water insoluble drugs due to cost of the later (66). Surfactants at low concentrations are allowed by regulatory agencies to enhance the solubility during dissolution testing of drugs that have poor aqueous solubility (67). Bile salt aggregates in the small intestine have a similar effect. Generally, the solubility of a drug is linearly related to the surfactant concentration, but this is not the case for the diffusivity of a drug-loaded micelle, which can be lower than the diffusivity of free drug (68).

When the surfactant solubility data file is created, the program calculates the CMC and solubilization enhancement factor based on the experimental solubility results. After fitting these parameters, the predicted surfactant solubility matched the experimental dataset and so did the predicted dissolution profiles (Table 2.4 and Figure 2.4B). According to the FDA, a drug is considered highly soluble when the highest dose is dissolved in 250ml of the medium. According to the solubility data, this would be the case for ritonavir (100mg) in phosphate buffer USP 6.8 + 0.25% SDS.

The Korsmeyer-Peppas model showed that the drug release was anomalous transport for the media without surfactant and Fickian diffusion controlled for the media with surfactant (69), however this model describes drug release from

polymeric systems. The Gompertz model which describes the drug release profile of immediate release tablets was therefore more suitable.

The drug dissolution–time profile of a poorly soluble drug observed in a single phase aqueous media is not representative of the in vivo situation due to the lack of partitioning kinetics (63). The human GI tract is composed of different segments with different pH values and medium composition. As Ritonavir moves along the GI tract it is expected to have a faster dissolution rate in the stomach (where the pH is low) and a lower dissolution rate and/or precipitation as the drug moves to the intestine where pH values are higher. In people with achlorhydria, the low level or absence of hydrochloric acid in the gastric secretions could represent a hindrance to the dissolution of weak bases such as ritonavir (70) and as reported for other weak bases (71).

In vitro two-tiered dissolution is one way to capture these in vivo aspects. It consists of two step dissolution test protocol with different pH values (pH 2 and 6.8), mimicking the passage of the drug along the gastro-intestinal tract. In vitro two-tiered dissolution tests are also appropriate to characterize the interaction between the drug dissolution rate, the degree of supersaturation, and the precipitation kinetics from different formulations (63). Several dissolution methods with pH change have been utilized to simulate the dissolution and transit of dosage forms from the stomach to the small intestine in vivo (72-77). The two-tiered dissolution tool present in DDDPlus^{TM} assess the effects of DF transit in terms of

pH change. However, the program uses the same medium composition for the two phases. If the surfactant were absent in the composition of the first phase, the fraction of the drug dissolved would be lower in the first phase as observed in measured values since no solubilization effects would occur and dissolution entirely depends on the API's ionization state.

The flowchart in Figure 2.6 is a provisional guide on how in vitro data can be used in combination with the DDDPlus™ software to enhance its predictive ability.

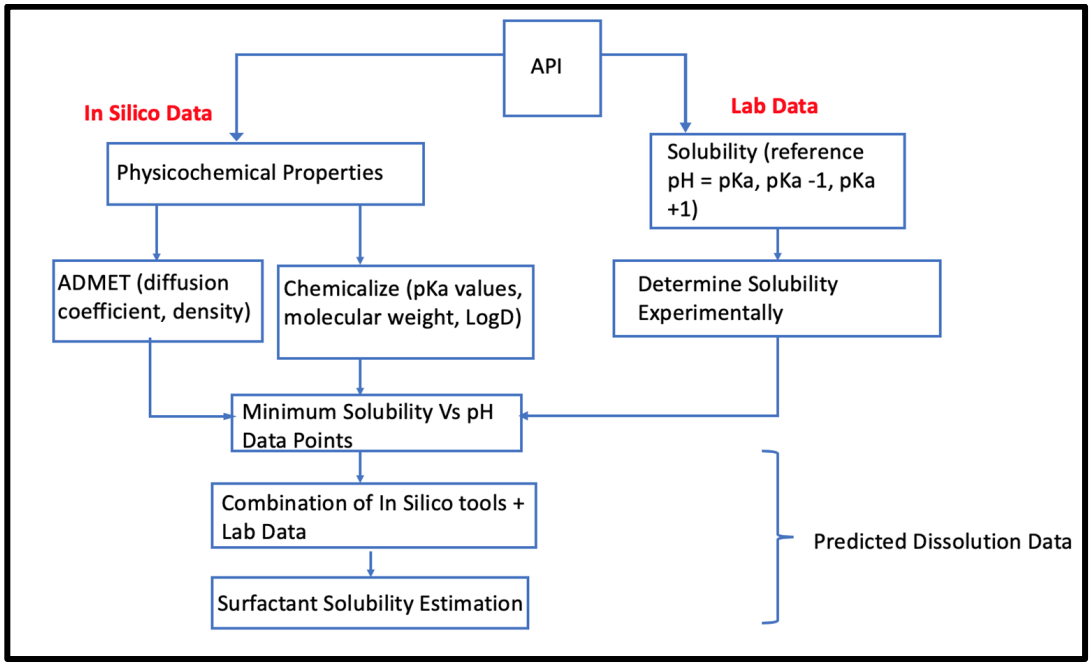


Figure 2.6 – A guide on the application of DDDPlus™ simulation software in early drug development

2.6 Limitations

Ritonavir has various pKa values as shown in Fig 1, however when the strongest acidic or basic pKa of ritonavir was used in simulations, a more precise solubility versus pH profile was obtained. For a drug molecule with various pKa values, the challenge is to find the suitable pKa values and solubility data points.

The program has provision for only one medium composition for a two-tiered dissolution as described in the USP 711 chapter and an option for pH input for the two phases in the "Dissolution Phase" window. There should be provision in the program to specify the composition of each medium of the two phases for a two-tiered replacement dissolution model. This would allow for evaluation of surfactant effect independently.

2.7 Conclusion

In order to utilize in silico methods to make accurate predictions on the dissolution profile, the solubility of a drug in relevant media has to be determined experimentally, data such as pKa, molecular weight, chemical structure can be obtained from databases or prediction software. The DDDPlus^{TM} software uses these data along with other data input from the ADMET predictor module such as diffusion coefficient, density to make predictions. When making predictions for media containing surfactants, the solubility of the drug in the media containing

different concentrations of the surfactant has to be determined experimentally and used as input for the surfactant model. Building the surfactant model is of utmost importance to obtain good predictions.

This study shows that the software is inadequate in making accurate and precise estimations without any external input. Its predictive ability can be improved with only a few laboratory experiments and external data. When used in this manner it can reduce the number of laboratory experiments required and can ultimately save time and costs especially in early drug development when there are limited API available and formulation decisions have to be made within a short timeline.

Chapter 3

Amorphous solid dispersions in early stage of formulation development: predicting formulation influence on dissolution profiles using DDDPlus™

This study is under review at Dissolution Technologies

3.1 Abstract

The objective of this study was to predict the effect of formulation strategies on the dissolution rate of a poorly soluble drug using computer simulations. Solid dispersion of ritonavir was prepared through hot melt extrusion. Dissolution test results of direct compressed tablets with and without disintegrant in various media with physiologically relevant pH were compared with simulations. Solubilizer and disintegrant effects were evaluated on the DDDPlus™ simulation software using previously published solubility data on ritonavir (78). Observed and predicted dissolution profiles similarity tests and drug release mechanisms were assessed. Optimization of the Solubilizer Effect Coefficient (SEC) on the program gives good estimations of the effect of copovidone in the extrudate on the dissolution profiles of all tablets. The SEC is dependent on the API's solubility at the local pH and the dissolved concentration of the solubilizer. Disintegrant concentration in the program has no effect on simulations, rather the disintegration time was the predictive factor. The mechanism of drug release was formulation controlled in the tablets without disintegrant and in the tablets with disintegrant was via drug diffusion and polymer surface erosion. DDDPlus™ has the potential to estimate the effect of excipients in a formulation on in vitro dissolution at an early stage in the drug development process. This could be useful

in decisions on formulation strategies to enhance bioavailability in BCS class II drugs.

3.2 Introduction

Crystalline solids are more commonly used in pharmaceutical formulations due to their chemical and physical stability. However, the crystalline property has negative effects on a drug's solubility and dissolution, especially for Biopharmaceutics Classification System (BCS) class II and IV drugs (79). Low solubility is a notable hindrance to the effective delivery of therapeutic agents because the absorption of orally administered drugs depends on dissolution and gastrointestinal permeability (80). The use of high-energy forms such as amorphous solid dispersions (ASDs) can improve drug solubility and consequently delivery. Poorly water-soluble drugs, when in the amorphous state tend to have higher solubility because no energy is required to break the crystal lattice during dissolution process (81).

Solid dispersions are systems where one component is dispersed in a carrier (usually a polymer and amorphous) and the whole system appears to be in a solid state (44). Solid dispersions have larger surface area, improved wettability and higher porosity, all of which hasten drug release (82). Hot Melt Extrusion (HME) is an established process for the manufacturing of solid dispersions which has been shown to improve wettability, flow properties and drug dissolution (45).

Solid dispersions of poorly soluble drugs require a polymer with some hydrophilic properties capable of forming intermolecular interactions with the drug (83). The polymer, copovidone (polyvinylpyrrolidone) was used as a solubilizer in this study to disperse ritonavir API (Active Pharmaceutical Ingredient) into a solid state formulation.

Ritonavir, the model drug used for this study is an HIV-1 protease inhibitor that inhibits the production of the structural and functional proteins of the HIV virus (84). It is poorly soluble at a high pH (400µg/mL in 0.1N HCl, 1µg/mL at pH 6.8, 37° C) and a substrate of the P-glycoprotein transporter (53,50).

DDDPlus™ (Dose, Disintegration and Dissolution Plus) is a software platform that models and simulates the in vitro dissolution of active pharmaceutical ingredients (API) and formulation excipients in various dosage forms under various experimental conditions (14). During drug development, in vitro dissolution testing is an important tool for evaluating candidate formulations and API interaction with excipients (14). There is an emerging trend in the industry to explore alternatives to dissolution testing and to apply them during product development to ensure product quality instead of relying on traditional dissolution testing (59). The use of DDDPlus for in silico predictions along with more traditional in vitro measurements was evaluated as part of the workshop titled "Dissolution And Translational Modeling Strategies Enabling Patient-Centric Drug Product Development", (59) held in May 2017 and attended by members

from worldwide regulatory agencies and consortia involved in drug development. Studies of drug-excipient interaction represent an important phase in the preformulation stage of dosage forms (85). The application of in silico methods to predict drug-excipient interaction and influence on formulation dissolution has the potential to expedite preformulation studies of new drugs.

The objective of this study was to assess formulation specific models in simulating drug – excipient interaction using DDDPlus^{TM}, by determining the impact of prediction factors in the program on solubilizer and disintegrant effect on the dissolution profile of an immediate release, poorly soluble drug. All in silico simulations were compared with in vitro measurements to confirm prediction accuracy. This strategy can be used in designing formulation strategies in early drug development with fewer laboratory experiments involved.

3.3 Materials

Ritonavir was provided by Abbvie Inc (Chicago, IL, USA). Microcrystalline cellulose (Avicel® PH-102 NF) was obtained from FMC Biopolymer (Philadelphia, PA, USA). Colloidal silicone dioxide was purchased from Cabot Corporation (Tuscola, IL, USA). Copovidone (Kollidon® VA 64) was purchased from BASF SE (Ludwigshafen, Germany). Croscarmellose sodium was purchased from PCCA Canada (London, ON, Canada). Magnesium stearate was obtained from H.L Blachford Ltd (Missisauga, ON, Canada). Hydrochloric acid (HCl) P.A

36.5% was obtained from Fisher Scientific (Fair Lawn, NJ, USA). HPLC grade water and water for the dissolution test media were generated in an Elgastat Maxima UF and an Elgastat Option 3B water purifier by ELGA Laboratories Ltd. (Missisauga, ON, Canada) and filtered through a Durapore® 0.22 µm GV filter by Millipore Canada Ltd. (Etobicoke, ON, Canada; for HPLC mobile phase). Acetonitrile for the HPLC mobile phase was purchased from VWR international LLC. (Radnor, PA, USA) and filtered through a Durapore® 0.45 µm HV filter by Millipore Canada Ltd (Etobicoke, ON, Canada).

3.4 Methods

The extrudate was prepared by melting copovidone and colloidal silicone dioxide at 150 ° C in a beaker placed in a silicone oil bath. Ritonavir was added to the molten excipients, mixed thoroughly at same temperature, and the mixture was cooled to room temperature. The composition of the resulting extrudate is shown in Table 3.1. The extrudate was ground in a mortar to powder form and stored in a dessicator. The powdered extrudate was used to prepare tablets with and without disintegrant (croscarmellose sodium) by direct compression at one metric ton pressure for 30 seconds and one minute respectively, using a Carver Laboratory Press by Fred S. Carver Inc Hydraulic Equipment (Manomee Falls, WI, USA). The composition of each tablet type is described in detail in Table 3.2.

Table 3.1 - Ritonavir extrudate Formulation

Ingredient	Amount (%)
Ritonavir	15
Colloidal Silicon Dioxide	1
Copovidone (PVP)	84

Table 3.2 - Ritonavir immediate release tablet composition with/ without disintegrant

Ingredient	w/ Disintegrant		w/o Disintegrant	
	Amount (mg)	% content	Amount (mg)	% content
Ritonavir Extrudate	100	11.57	80	80
Microcrystalline Cellulose (Avicel ph-102 NF)	586.67	67.87	19	19
Croscarmellose Sodium	174.89	20.23	-	
Magnesium Stearate	2.86	0.33	-	
Colloidal Silicon Dioxide	-	-	0.5	0.5
Sodium Stearyl Fumarate	-	-	0.5	0.5

3.4.1 Solubility and Dissolution Testing

The solubility of the extrudate was determined via the shake flask method. 5 mg of the extrudate was added to 5 mL of each medium (0.1M HCl, 0.01M HCl and phosphate buffer USP 6.8), the solution was placed in a shaker by Heraeus Instruments Inc. (USA) for 72 hours at 25° C. Samples (1 mL) were withdrawn without replacement at each time point (24, 48 and 72 hours) and centrifuged at 15,000 rpm. The supernatant (500 µL) was withdrawn and transferred into 2.5 mL vials for HPLC analysis.

The pH of the media was measured using an Accumet ® XL 20 pH-meter by Fisher Scientific (Fair Lawn, NJ, USA). The media was deaerated by filtration, ultrasound and vacuum. The dissolution testing was performed using a VK 7020 system from Varian Inc. (Cary, NC, USA) equipped with 70 µm Full Flow™ filters (Varian Inc.) and a VK 8000 auto sampler (Varian Inc). Dissolution tests were performed with USP Apparatus 2 and 900 mL dissolution medium (hydrochloric acid 0.1M, 0.01M and phosphate buffer USP 6.8) at 75 rpm rotation speed. Samples (1.0 mL) were withdrawn in triplicate without replacement at each time point (3, 5, 10, 15, 20, 30, 45, and 60 minutes) for HPLC analysis.

3.4.2 HPLC Analysis

A previously published method was used (48). In brief, a calibration curve was prepared for a range from 3.75% to 120% of the expected maximum drug concentrations in each medium and the correlation coefficient (R^2) for the calibration curve was ≥ 0.998. A VP-class Shimadzu Instrument (Kyoto, Japan) liquid chromatograph (the analytical column was a Lichrospher®60 RP Select B (5 µm, 12.5x4 mm, by Merck Darmstadt, Germany) column) composed of a CBM-20A system controller, two LC-10AS pumps, an SIL-10ADVP autosampler and an SPD-M10AVP diode array detector was used for the analysis. The mobile phase (acetonitrile, water and trifluoracetic acid 57:43:0.1 (v/v/v)) was deaerated before use, using a combination of vacuum filtration and ultrasound and the flow rate was set to 1 mL/min. An injection volume of 50 µL was used without dilution and the retention time for ritonavir was four minutes approximate with a total run time of eight minutes. A wavelength of 240 nm was selected for the analysis.

3.4.3 DDDPlus™ Simulation

DDDPlus™ (Dose, Disintegration and Dissolution Plus) version 5.0.0011 by Simulations Plus Inc (Lancaster, CA, USA) is a software program that simulates the dissolution behavior of different formulations by defining excipients and test

conditions. There are three main tabs in the software – formulation, dissolution method and simulation tabs (48).

In this study, the software was used to predict the active ingredient – excipient interaction in the formulation. The formulation composition was defined by selecting all the ingredients and their functions from the included database. The IR:Tablet dosage form was selected for all simulations. The physical dimensions and manufacturing properties of the tablets consistent with the tablet compression process were entered into the software platform. Previous studies by Njoku et al (2019) showed that a solubility vs pH profile can be created by the program from a drug's experimentally determined solubility using known pKa and other physicochemical properties of the API. Also, one data point of measured solubility was found to be sufficient to create a solubility vs pH profile for simulations, therefore the solubility of ritonavir API was determined experimentally to create a solubility vs pH profile. The solubility of ritonavir drug powder at pH 1.0 (0.57 mg/mL) (78) was used as the reference solubility. The solubilizer constant for the solubilizer, PVP, was calibrated to fit the concentration of solubilizer in the tablet (optimization) in order to estimate the effect of a different concentration of the solubilizer within the formulation on the dissolution profile. This constant empirically describes the interaction between the solubilizer and the active ingredient. The optimization module in the program was used to build the formulation specific model. The formulation-specific model can

be used to estimate the probable changes in dissolution when excipient content and experimental parameters are varied. DDDPlus models the effect of solubilizers on ingredients solubility using the equation:

$$S_e = S_{API}(pH) \times \left[1 + \sum k_{SE,i}\, C_{D,i}\right]$$

Equation 3.1

Where S_e is the solubility of the extrudate, $S_{API}(pH)$ is the active drug's solubility at the local pH without solubilizer, $k_{SE,i}$ is an optimizable coefficient for the i^{th} solubilizer called the Solubilizer Effect parameter (units of L/mg), and C_D is the dissolved concentration of the i^{th} solubilizer.

The dissolution parameters were defined according to the test conditions; USP apparatus 2 (paddle), 900 mL medium, 75 rpm rotation speed and three different medium types (HCl 0.1M, HCl 0.01M, and USP phosphate buffer 6.8).

Single simulations were performed for each in silico experiment using 60 minutes as the length of simulation, consistent with the experimental design. The predictions of the dynamic dissolution from DDDPlus were compared to the in vitro results.

3.4.4 Statistical Methods

DDSolver, an add-in in Microsoft Excel™ designed for dissolution profile data analysis such as profile comparison or modeling, was used to evaluate and compare between in vitro and in silico results. Observed and simulated dissolution profiles were compared using the f2 statistical test for similarity. Only percent dissolved values less than 85% were chosen for the similarity test. For cases where most values were above 85%, the lowest four values were chosen. The Korsmeyer-Peppas model in DDSolver was used to determine the drug release mechanism. Only percent dissolved values less than 65% were chosen for the Korsmeyer-Peppas model fitting and in cases where most of the values were above 65%, the first three values were used. The first order, zero order, Gompertz and Hopfenberg models were also evaluated using the DDSolver.

3.5 Results

Solubility tests on ritonavir extrudate confirmed the pH-dependent solubility of ritonavir as shown in Table 3.3. There was an improvement on the solubility of ritonavir due to excipient (solubilizer) effect.

Table 3.3 - Ritonavir and extrudate solubility comparison in different media

Media	API Solubility (mg/ml)*	Extrudate Solubility (mg/ml)
0.1M HCl (pH 1)	0.57	0.96
0.01M HCl (pH 2)	0.01	0.31
Phosphate buffer USP 6.8	0.002	0.06

***Ritonavir solubility was measured by Njoku et al, 2019**

The dissolution tests result of the tablets with disintegrant are shown in Figure 3.1. Predictions showed similarity to observed values in all media. There was a reduction in the fraction dose dissolved at 20 minutes in the medium of pH 2 and at 15 minutes in the medium of pH 6.8. This could be attributed to precipitation of the crystalline drug due to drug supersaturation at this pH where ritonavir has a lower solubility (86). The similarity factor (f2) between observed and predicted profiles is shown in Table 3.4.

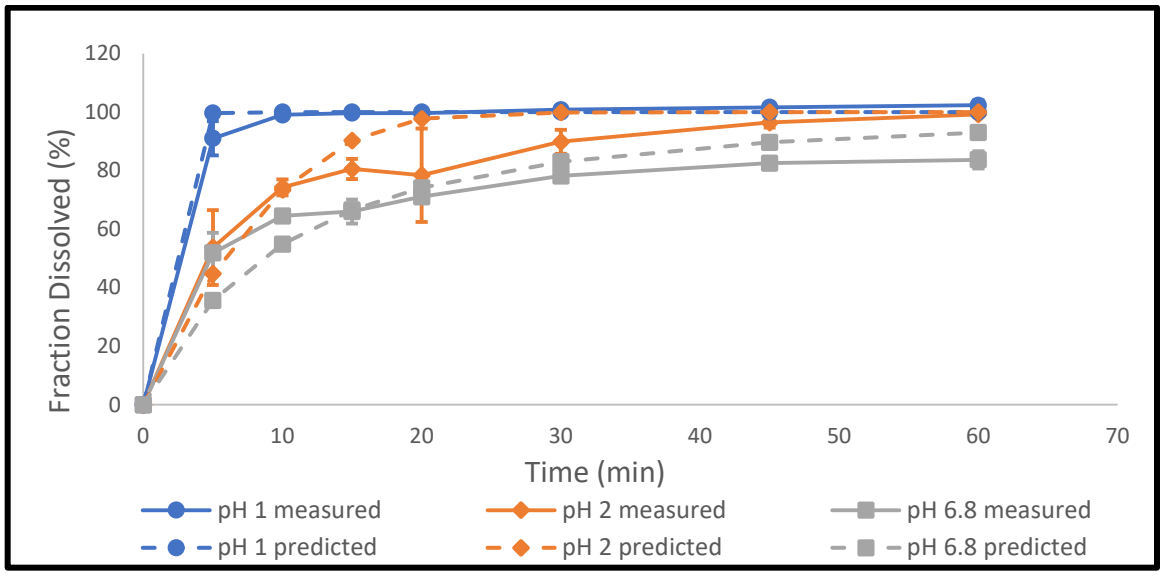


Figure 3.1 - Dissolution of ritonavir extrudate 100mg tablets with disintegrant in different media and simulated profiles.

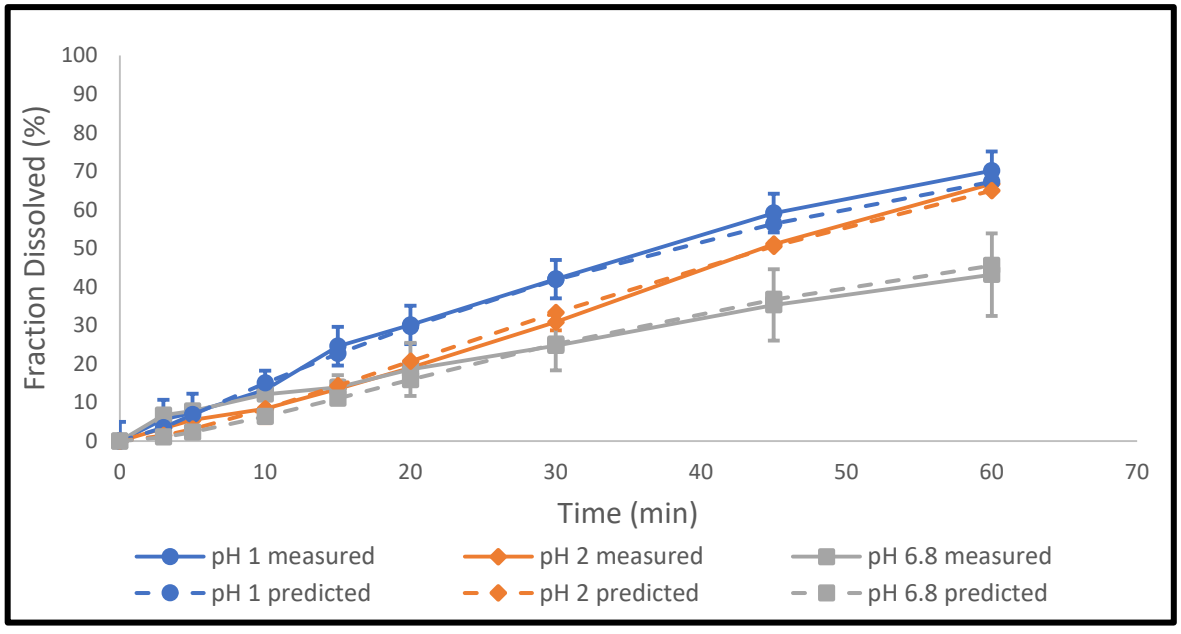


Figure 3.2 - Dissolution of ritonavir extrudate 80mg tablets without disintegrant in different media and simulated profiles.

The dissolution test results of the tablets without disintegrant are shown in Figure 3.2. Predictions showed a high similarity with observed values in all media used.

Table 3.4 – Comparison of in silico to in vitro data

Dissolution Profile	f2 test (accepted?)	R^2
Tablet with disintegrant dissolution at pH 1.0	73 (yes)	0.88
Tablet with disintegrant dissolution at pH 2.0	52 (yes)	0.74
Tablet with disintegrant dissolution at pH 6.8	54 (yes)	0.78
Tablets without disintegrant dissolution at pH 1.0	85 (yes)	0.99
Tablets without disintegrant dissolution at pH 2.0	87 (yes)	0.99
Tablets without disintegrant dissolution at pH 6.8	71 (yes)	0.9

The Korsmeyer-Peppas model developed by Korsmeyer et al., (1983), is expressed as:

$$f_t = Kt^n$$

Equation 3.2

Where, f_t is the fraction of the drug released at time t, K is a release rate constant, and n is the exponent of release. Plotting logarithms of fraction dissolved versus logarithm of time, helps estimate a value of n, which can be used to identify mechanisms of dissolution. Analysis of the Korsmeyer-Peppas equation with the data resulted in n-values of 0.086, 0.362, 0.221 (tablets with disintegrant) and 0.885, 1.177, 0.733 (tablets without disintegrant) at media with pH 1, pH 2 and pH 6.8 respectively (Table 3.5). This indicates that the drug release from the tablets with disintegrant (with n values < 0.43) was controlled by Fickian diffusion (48,88). After model fitting with DDSolver, tablets with disintegrants had good fits (R^2_{adj} = 0.894, 0.775 and 0.701) for a first order model and Gompertz model which describes drug release from systems where the release rate is concentration dependent. All of this suggest that the drug release for tablets with disintegrant was governed by Fickian diffusion. The tablets without disintegrant resulted in n-values (Korsmeyer-Peppas eq.) which were higher or equal to 0.89, which suggested a non-Fickian release mechanism. These tablets also showed good fits (R^2_{adj} > 0.93) for the zero order and Hopfenberg models which indicates that without disintegrant, the drug is released via surface erosion of the polymer and is therefore controlled by formulation factors.

Table 3.5 – Korsmeyer-Peppas equation n – values, R^2_{adj} results and SEC values for tablet dissolution under various conditions

Dissolution Profile		n - value	R^2_{adj}	SEC
Tablet with disintegrant	pH = 1.0	0.086	0.803	0.73
	pH = 2.0	0.362	0.920	3.51
	pH = 6.8	0.221	0.819	14.99
Tablets without disintegrant	pH = 1.0	0.885	0.993	0.53
	pH = 2.0	1.177	0.996	0.22
	pH = 6.8	0.773	0.991	4.25

The parameter SEC which estimates the interaction effect of the solubilizer (copovidone) on the dissolution of the extrudate was calibrated for each dissolution condition. The results of this calibration are shown in Figure 3.3, where it can be seen that the solubilizer has a more pronounced effect for situations not conducive to dissolution of the extrudate, i.e. absence of disintegrant and higher pH. The influence of the SEC is also more variable for cases with higher pH due to slower dissolution.

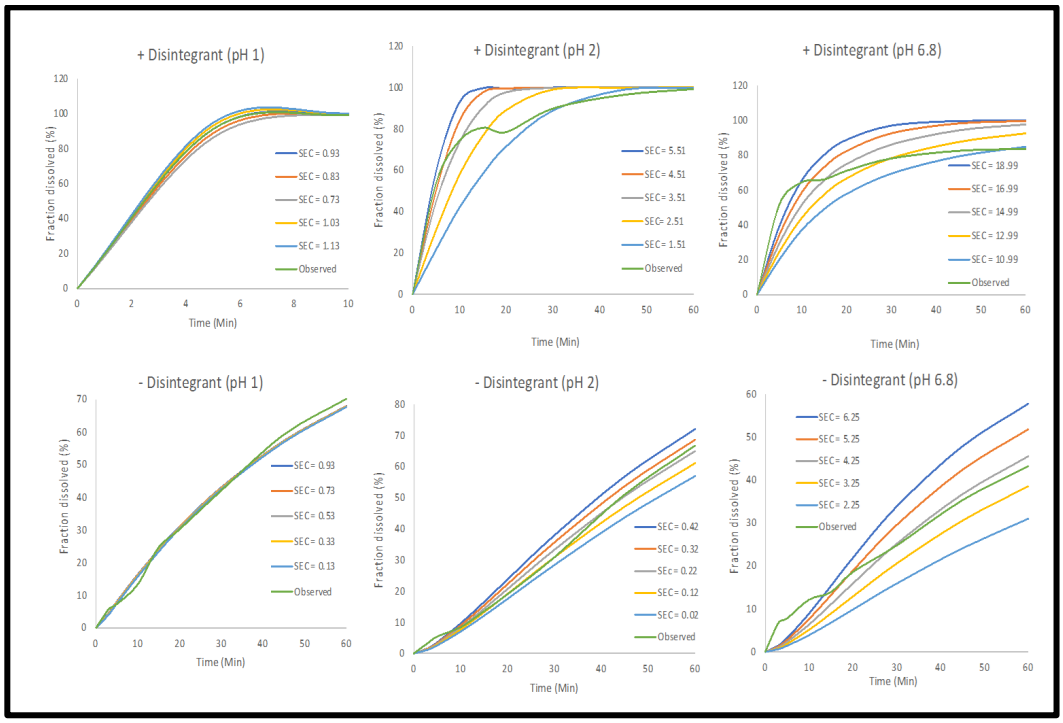


Figure 3.3 – Comparison of observed dissolution profiles with predicted dissolution profiles with different values of the Solubilizer Effect Coefficient (SEC)

3.6 Discussion

The polymer matrix carrier in which the active pharmaceutical ingredient (API) is homogenously dispersed contains excipients which are capable of controlling the drug release rate. The shear mixing of the molten mass during preparation of the extrudate causes dispersion of the drug into the polymer matrix at a molecular level along with the possibility of drug-polymer interactions (89). The excipient which is the rate-controlling material can be water-soluble or swellable

(hydrophilic matrix) such as polyvinylpyrrolidone (used in this analysis) or water-insoluble (hydrophobic or inert matrix) (90). The rate at which a drug is released from the swellable hydrophilic matrix is determined by processes such as hydration of the polymer that leads to swelling, diffusion of the drug through the hydrated polymer, drug dissolution and polymer erosion (91). These processes occur simultaneously to facilitate drug release. The factors which influence drug release in hydrophilic matrices such as extrudates are the drug solubility, polymer viscosity, drug/polymer ratio, amount of water entering the matrix and compression force (92).

Embedding the drug in a complex matrix usually delays the onset of dissolution of immediate release tablets. Disintegrants should therefore be added to the formulation to promote the breaking up of the tablet into small granules and constituent particles leading to faster liberation of the drug particles from the tablet matrix resulting in an increased surface area for subsequent dissolution (93). Copovidone has high binding and gelling properties; hence, when present in large amounts in the solid dispersion, it can result in an increased disintegration time of tablets (94). For this reason, when a high concentration of disintegrant (20% of croscarmellose sodium was used in the tablets, the disintegration time was significantly decreased. The tablets without disintegrant had a prolonged disintegration time, lasting over 60 minutes. The dissolution process of the extrudate tablets was formulation controlled, however the presence of disintegrant

in some of the tablets enhanced drug release, and in those tablets, dissolution was controlled by the extrudate particle properties due to fast and complete tablet disintegration (48). The suggested mechanisms of drug release for the two tablet types are summarized in Figure 3.4.

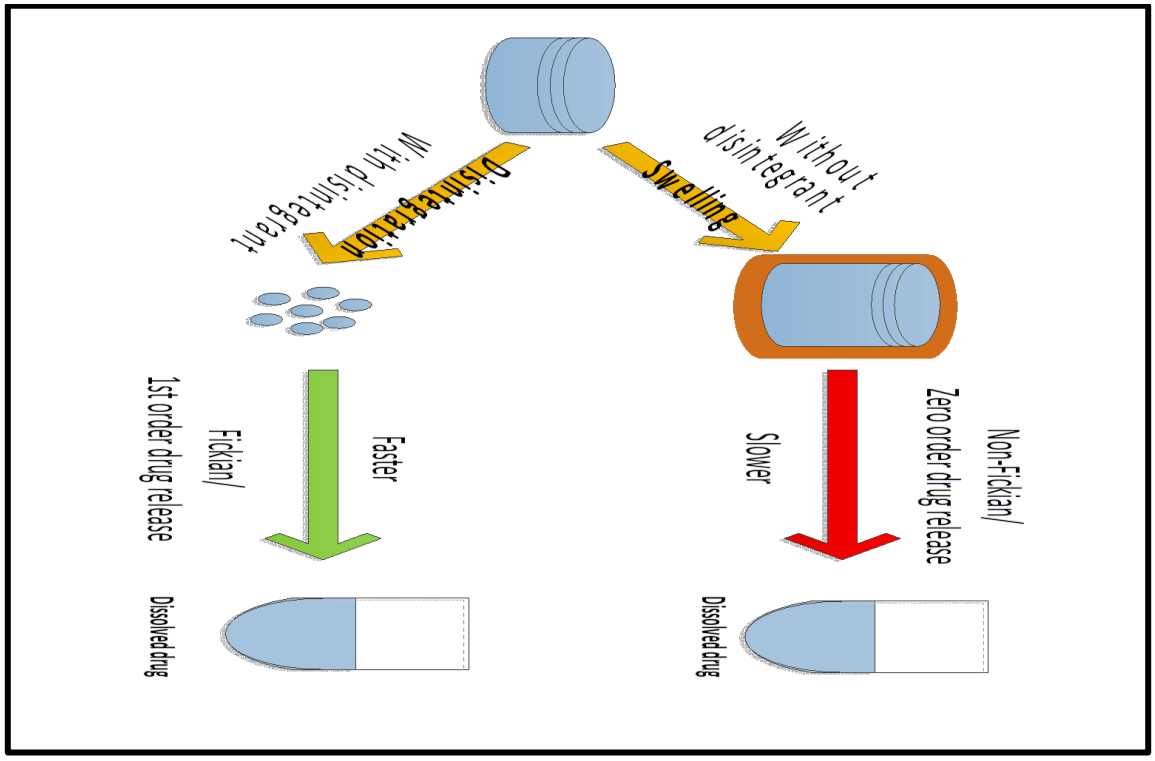


Figure 3.4 – Probable dissolution mechanisms based on mechanistic understanding of the processes

The Mass Transfer Model uses an empirical relationship that accounts for solubilizer effect on the dissolution rate. In this model, calibrating the solubilizer effect coefficient for one solubilizer amount will provide estimations of the

effects of differing amounts of the same solubilizer on the active ingredient's dissolution. A change in the amount of the drug or polymer results in a different solubilizer effect parameter and consequently a different dissolution profile. The solubilizer effect parameter has an inverse relationship with the drug/polymer ratio. If the drug/polymer ratio is low, the solubilizer effect is enhanced and higher percentage of the drug is dissolved. Tablets with and without disintegrant had different solubilizer effect coefficient because although the drug/polymer ratio in both tablets were the same, the overall concentration of solubilizer in the tablets were different and the tablets had varying solubility depending on the media. Also, the dissolved concentration of the solubilizer (C_D) was influenced by the difference in disintegration time between the two tablets which in turn was impacted by the higher polymer concentration and absence of disintegrant in the tablet without disintegrant. F2 test results for similarity showed that calibration of the solubilizer effect for all tablets produced simulations with acceptable predictive accuracy (Table 3.4). The amount of the disintegrant has no effect on simulations in the program if the IR: Tablet dosage form option is selected, however the disintegration time is an important factor in estimating the rate of drug release especially in the early time points.

3.7 Conclusion

Simulation of dissolution profiles for immediate release and controlled release tablets involves choosing the appropriate dosage form in the program, input of disintegration time, API solubility vs pH and optimization of excipient effect. DDDPlus simulation software, when used with the right data, can be used in determining formulation strategies during early drug development due to its ability to predict the effect of an excipient on API solubility and dissolution rate if the excipient is identified on the software and the excipient effect is optimized. Prediction of excipient influence on the dissolution profile of a drug using DDDPlus involves quantifying the interaction between the active ingredient and the excipient. The solubility of the active ingredient in the media for dissolution has to be determined experimentally and the tablet dimensions have to be entered in the program. Other physicochemical parameters of the drug such as its molecular weight, pKa, LogD which are required in DDDPlus can be obtained from existing data which is typically present during the drug development process. The API's solubility in the dissolution medium has to be determined experimentally. The function of each excipient has to be selected in the software as the program has empirical relationships that define each function. The influence of the excipient on the active ingredient's solubility has to be defined and enhanced through optimization. It was found that a combination of these methods can achieve acceptable predictions of dissolution profiles which

compared well to in vitro measurements. The use of this in silico tool, in this manner can assist in decisions concerning the choice of suitable excipients to be used in the formulation. It can reduce the number of laboratory experiments that are typically needed to study drug-excipient interaction and thus can shorten the overall time frame of the formulation development process.

Chapter Four

Discussion, Conclusion and Future Directions

4.1 Discussion

Oral pharmaceutical solid dosage forms, such as tablets and capsules, are one of the most predominant form to administer drugs to patients. As described by the USP, the performance of the drug is influenced by the disintegration and dissolution behavior of the solid dosage form. The disintegration process is especially critical for immediate-release dosage forms. The next step in the sequence of the drug's journey towards bioavailability is the dissolution process. Dissolution testing is a standardised method for measuring the rate and extent of drug release from a given dosage form. It is a requirement for all solid oral dosage forms and is used throughout the development and finished product stages for product release and stability testing (22). For an oral dosage form to be therapeutically effective, the active pharmaceutical ingredient (API) must be dissolved in solution and then absorbed into the systemic circulation to facilitate its transport to site of action. This process affects the overall bioavailability of the API. Drug dissolution involves two steps; the drug release from the dosage form (liberation process) and the drug transport within the dissolution medium (convection process) (95). Several factors influence dissolution and they include;

i. The physicochemical properties of the drug:

In this study the solubility of the drug was experimentally determined. The molecular properties were obtained from the online resource chemicalize.com,

particle size and diffusivity in the dissolution medium were estimated by the ADMET Predictor™ module in DDDPlus™.

ii. Formulation characteristics of the dosage form:

The effect of the excipients in an extrudate formulation was taken into account in simulations. The manufacturing parameters of the tablet were entered into the software for simulations.

iii. The dissolution method:

The apparatus type, the volume of the dissolution medium, surface tension, ionic strength, viscosity, the pH of the medium and hydrodynamic conditions all have an impact on the rate and extent of dissolution (95).

In early drug development, in vitro dissolution testing can be used in evaluating API and determining the appropriate formulation strategies for suitable drug candidates. It is useful in evaluating possible risks such as food and excipient effects on bioavailability (in controlled release dosage forms such as the ritonavir extrudate tablets in chapter 3).

In silico methods have been previously used to predict drug solubility (96-98). Liao and Nicklaus (2009) compared programs predicting pKa values of APIs and reported that the ADMET Predictor™ ranked fourth compared with eight other programs that were studied. Hewitt et al (2009) studied the predictive capability of commercial solubility models such as the ADMET Predictor™ and found that none of the models were able to predict solubility accurately, this was also

observed in this study as the pKa values sourced from the ADMET Predictor™ failed to give an accurate solubility vs pH profile.

Identification of the number of experimental solubility vs pH test data points that would be needed to create a solubility profile for a drug that can be used to predict its dissolution in various media that represent the physiological pH range of the gastrointestinal tract was a critical step in the study because the reference solubility of the API in the medium pH (C*s*) is a fundamental basis on which the dissolution mass transfer model utilized by the software is built and the media chosen for analyses should all have a pH which is reflective of what is obtainable in vivo.

Ritonavir was chosen as the model drug for this study because it is poorly water soluble, analyses of the dissolution behavior of a poorly soluble drug using in silico tools will assist developers when working with new chemical entities.

The Biopharmaceutics Classification System (BCS) as described in Chapter 1 classifies an API based on the solubility and permeability of the drug (6), and depending on the class of the drug, an in vitro dissolution study can provide a basis for a BCS-based biowaiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms. There are challenges when selecting appropriate dissolution media for poorly water-soluble drugs, such as classes II and IV drugs that are poorly soluble, that will be capable of discriminating between drug products (19). Different approaches have been

suggested to overcome this issue, some involve using a large amount of the dissolution medium (100,101), a co-solvent method to increase drug solubility and the use of surfactants to improve drug solubility (101-103). Of all the methods investigated, the use of media containing artificial surfactants was proposed as a suitable method because of the presence of various biorelevant surfactants in the gastrointestinal fluid such as bile salts, lecithin, cholesterol and its esters (6,19). Also studies by Park et al (2006) show that the class of surfactant used in the dissolution medium plays a role as well, the dissolution of poorly soluble acidic drugs were more enhanced when cationic surfactants were used, likewise in this study, the anionic sodium lauryl sulphate greatly improved the dissolved percentage of the poorly soluble basic ritonavir. The DDDPlus™ program will account for the effect of micellar solubilization on the dissolution profile of the drug in media containing surfactant if the concentration of the surfactant in the media is above its critical micelle concentration, however it overestimates the extent of dissolution to be 100%. To create predictions that were closer to the observed values, the surfactant model was built in the program by optimizing the Solubility Enhancement Factor (the SEF is an optimizable parameter that is dependent on the surfactant concentration in the medium, the critical micelle concentration of the surfactant and the API solubility in the medium), and calibrating this parameter to experimental surfactant solubility

values, subsequent simulations showed that the program was then able to give suitable and useful predictions.

Matsui et al in 2016 were able to distinguish between the pharmacokinetic profiles of two different oral dosage forms of Itraconazole using a multicompartmental in vitro dissolution apparatus, gastrointestinal simulator consisting of three chambers mimicking the upper gastrointestinal tract. Their studies also showed that improved drug dissolution by formulations results in enhanced permeation of the drug through cell monolayer. Dynamic dissolution systems such as the artificial stomach-duodenum model aimed at replicating the dynamic aspects of in vivo dissolution have been used to evaluate gastric emptying effect on drug dissolution and the supersaturation-precipitation propensity of weak bases during transfer from a more soluble acidic gastric compartment to a less soluble duodenal compartment of higher pH (105-109). The DDDPlus program was unable to differentiate the two phases as two distinct compartments whose composition could be defined separately in the two-tiered dissolution transfer model. Development of a multicompartment phase system in the program will assist in mimicking the dynamic aspects of in vivo drug dissolution.

Ritonavir is commercially available as a marketed product, Norvir tablet (an amorphous solid dispersion containing 100 mg of ritonavir prepared by hot melt extrusion (50). In this formulation, ritonavir is present in the same concentration

as the extrudate in this study, as a 15% drug load (w/w) with copovidone as the water-soluble polymeric carrier and sorbitan monolaurate as the surfactant (110). In their study, Ellenberger et al (2018) observed similarity in dissolution behavior and bioavailability between prepared amorphous solid dispersion and the reference tablet dosage form, Norvir.

The aqueous solubility of ritonavir at pH 6.8 was observed to be 0.002 mg/ml, which suggests that to dissolve the lowest available dose of 100 mg, approximately 50 L of media will be required which is not obtainable in vivo. Therefore, formulation strategies such as solid dispersion may to be employed to tackle this challenge. The crystalline form of a drug has the advantage of high purity and physical stability, but the lattice energy barrier is a major constraint in the dissolution of crystalline drug molecules (111). The amorphous state has a disordered structure compared to the crystalline state and possesses higher free energy which leads to higher apparent water solubility and dissolution rate as observed in this study (112,113). Hence, amorphous solid dispersions have been developed to be kinetically stabilized and to retain the solubility advantage of the system (112).

The dissolution profile of the ritonavir extrudate was estimated by taking into account all the ingredients in the formulation and optimizing the Solubilizer Effect Coefficient (SEC) which is a constant that predicts the solubility of the extrudate based on the solubility of the API in the medium and the dissolved

concentration of the solubilizer. The SEC constant was found to be more pronounced in conditions not favorable for dissolution of the extrudate, such as a higher medium pH and less concentration of the solubilizer.

Overall, the influence of formulation, sink conditions, surfactant and medium pH on dissolution behaviour and the discriminatory effect of dissolution testing was evaluated using a combination of in vitro and in silico tools to assess the predictive power and utility of the software program DDDPlus™.

The dissolution market is currently valued at over $160 million and is expected to grow by at least 4% annually over the next three years. In 2017, basic and applied research and development accounted for over 55% of the demand for dissolution testing as shown Figure 4.1 (114). Early drug discovery and development takes at least 5 years while decisions on formulation strategies and BCS classification may take up to 6 months of time spent on drug development. When only solubility testing of the limited API available at this stage is required as this study has shown, to predict the dissolution behavior of a drug, this time and cost can be reduced.

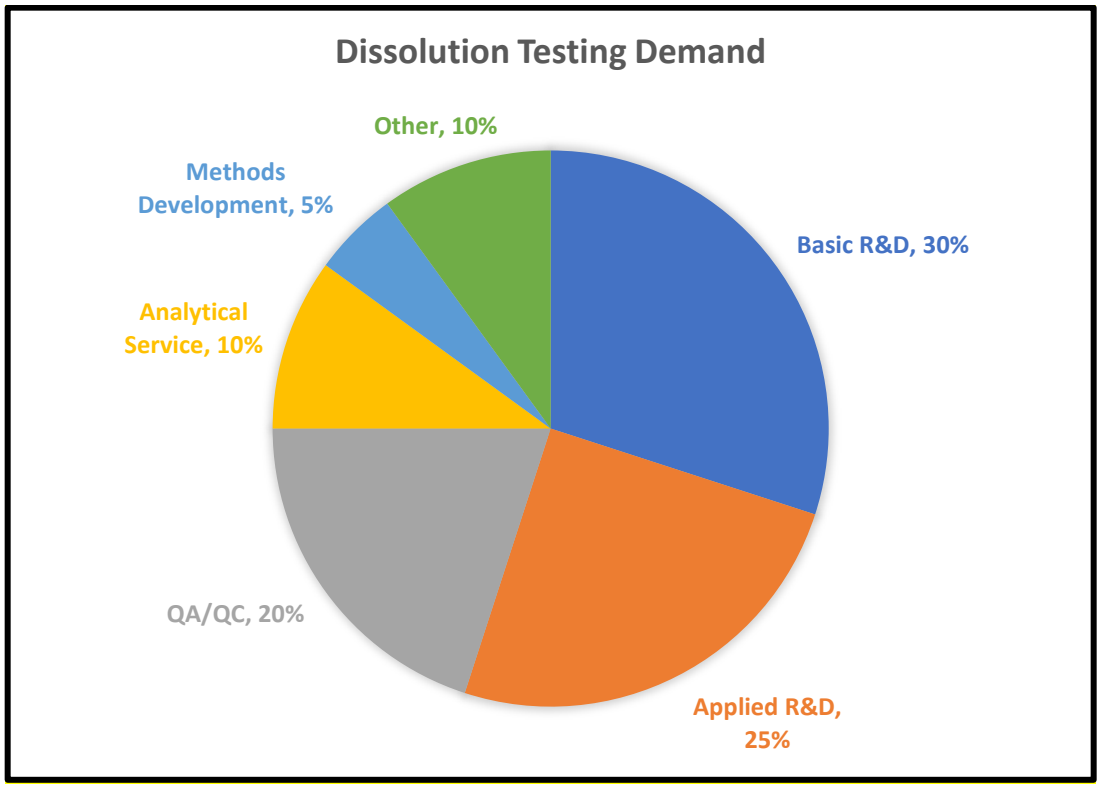


Figure 4.1 - Dissolution testing demand by function in 2018. Basic R&D - the discovery of fundamental properties and scientific principles. Applied R&D – product development and improvement. QA/QC – raw materials and production control. Analytical service – general services or contract services. Methods development – SOP development and improvement. Other – Educational and other uses. (data from Ref. 114)

4.2 Conclusion

A mechanistic study of the factors impacting dissolution testing is imperative to create models during simulation that will adequately reflect in vivo drug dissolution conditions.

This research has demonstrated that a few in vitro solubility tests involving minimal amounts of the active pharmaceutical ingredient, along with estimates of the physicochemical properties of the drug inputted into the simulation software can give a basic understanding of dissolution behavior.

The surfactant model in the software program gave good predictions, the program was also able to predict the effect of excipients in a formulation when used in the manner outlined in the study. At the preclinical exploratory stage, estimation of dissolution profiles that have a similarity to actual in vitro test profiles is both acceptable and beneficial, since at this stage only a basic understanding of dissolution behavior is required for in vitro characterization and decision on formulation technology to overcome low solubility and dissolution rate limitations in new chemical entities.

The principles of this study can also be useful in Quality by Design (QbD) at the later stage in development when more data on the drug product is available. This study showed that the program is not sufficient in itself in making predictions but require a few in vitro solubility tests, as in silico models require “high-quality” data, the predictive quality of the model is only as good as the dataset provided on solubility.

4.3 Future Directions

Polymorphic forms of a drug can have different chemical and physical properties including melting point, apparent solubility and dissolution rate. These properties can affect drug product stability, bioavailability and consequently the quality, safety and efficacy of the drug product (115). The effects of API polymorphism on dissolution profiles is an API parameter which is subject to change and it should be studied especially for poorly soluble drugs. The FDA recognized the importance of polymorphism in its guidance issued in July 2007 where it states "For a drug whose absorption is only limited by its dissolution, large differences in the apparent solubilities of the various polymorphic forms are likely to affect BA/BE. On the other hand, for a drug whose absorption is only limited by its intestinal permeability, differences in the apparent solubilities of the various polymorphic forms are less likely to affect BA/BE. Furthermore, when the apparent solubilities of the polymorphic forms are sufficiently high and drug dissolution is rapid in relation to gastric emptying, differences in the solubilities of the polymorphic forms are unlikely to affect BA/BE." This infers that polymorphism is critical for poorly soluble BCS class 2 and 4 drugs (116). In 1998, Norvir semi-solid capsules supplies were challenged by a new much less soluble crystal form of ritonavir. The less soluble polymorph form II with a "cis" conformation has a more stable packing arrangement and studies by Bauer et al in

2001 indicated that its appearance may have been as a result of a coincidence of a highly supersaturated solution and a heterogenous nucleation by a degradation product. Varying the concentration of the polymorphs in a dosage form and assessing the resulting effect on its dissolution profile is a promising area of research as many compounds have polymorphs with different solubilities and stability.

The possibility of estimating a two-tiered dissolution profile by using the program to simulate a multi-compartment transfer system and evaluate the dissolution and precipitation of a weakly basic drug during transfer from the stomach to the small intestine should be explored. Simulation of a biphasic dissolution-partition test method in aqueous media and an organic phase for BCS class II drugs could also be developed for establishing in vitro-in vivo relationship (118).

The in silico tools used in this study can also be applied to predict the dissolution behavior of drugs with published in vitro dissolution data to further assess the software program's capabilities.

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