Predictive in vitro dissolution tools : application during formulation development

Emmanuel Scheubel

University Clermont-Ferrand 1 Faculty of Pharmacy
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by

Scheubel Emmanuel

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Chairman:	Professor Gilles Ponchel	(Faculty of Pharmacy, University of Paris-XI, France)
Jury:	Professor Jean-Michel Cardot	(Faculty of Pharmacy, Clermont-Ferrand, France)
	Professor Eric Beyssac	(Faculty of Pharmacy, Clermont-Ferrand, France)
	Doctor Laurent Adamy	(Galenical and Analytical Development, F.Hoffmann-La Roche Ltd, Basel, Switzerland)
Reviewer:	Professor Philippe Maincent	(Faculty of Pharmacy, Nancy, France)
	Doctor Johannes Krämer	(Phast, Germany)

Equipe de Recherche Technologique
« Conception, Ingenerie et Development de l'Aliment et du Médicament » (ERT CIDAM)
Faculté de Pharmacie – CNRH Auvergne – IFR Santé - Université d'Auvergne

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I dedicate this work to my family and in particular to

Hugo

and

Jade

About Roche

The experimental parts included in this thesis were carried out at the Pharmaceuticals Division of Galenical and Analytical Development (PTDFA) at F.Hoffmann-La Roche Ltd, Basel, Switzerland.

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Table of Content

Abbreviation

API	Active Pharmaceutical Ingredient
BA	Bioavailability
BE	Bioequivalence
BCS	Biopharmaceutical Classification System
CQA	Critical Quality Attribute
DoE	Design of Experiment
DP	Drug Product
DR	Dissolution Rate
DS	Drug Substance
EIH	Entry into Human. Corresponds to phase 1 of the development of new medicine.
FDA	Food and Drug Administration
FaSSGF	Fasted State Simulated Gastric Fluid
FaSSIF	Fasted State Simulated Intestinal Fluid
FeSSIF	Fed State Simulated Intestinal Fluid
GIT	Gastro Intestinal track (GI tract)
ICH	International Conference on Harmonization
IP	Intellectual properties
IR	Immediate Release
IVIVC	In Vivo In Vitro Correlation
IVIVR	In Vivo In Vitro Relationship
JP	Japanese Pharmacopoeia
MR	Modified Release
PAT	Process Analytical Technology
PE	Pharmacopeia European
PoC	Proof of Concept
PSD	Particle Size Distribution
QbD	Quality by Design
QC	Quality Control
USP	United Stated Pharmacopeia
XRPD	X-ray powder diffraction

Glossary - Definition of terms

Bioavailability : Bioavailability is defined as the relative fraction of a drug dose that enters the systemic circulation.

Bioequivalence : Bioequivalence of a drug product is achieved if its extent and rate of absorption are not statistically significantly different from those of the standard when administered at the same molar dose.

Biowaiver : The regulatory acceptance of in vitro testing as a reliable surrogate for an in vivo bioequivalence study is commonly referred to as biowaiver.

Input profile : In vivo dissolution or in vivo absorption (includes permeability and dissolution phases) of the drug from a particular dosage form

Sink condition: The term sink conditions is defined as the volume of medium at least greater than three times that required to form a saturated solution of a drug substance. It is a mandatory working condition for QC dissolution testing.

List of original papers

This thesis is based on the following papers and posters, which are referred to in the text by their respective numerals (1 to 4).

Paper 1.

E. Scheubel, V. Hoffart and J-M Cardot. **Selection of optimal API properties using in vitro dissolution, animal study and IVIVR to derisk Human study during development**. (2010) *not submitted*

Paper 2

E. Scheubel, L. Adamy, E. Beyssac and J-M Cardot. **Selection of the Most Suitable Dissolution Method for an Extended Release Formulation based on IVIVC level A obtained on Cynomolgus Monkey** (2010). Drug Development and Industrial Pharmacy, Vol. 36, No. 11 , Pages 1320-1329

Paper 3

E. Scheubel, M Lindenberg, E. Beyssac and J-M Cardot. **Small Volume Dissolution Testing as Powerful Method during Pharmaceutical Development**. (2010) *Pharmaceutics*, *2*, 351-363

Poster

Nicole Wyttenbach1, André Alker, Olaf Grassmann, Emmanuel Scheubel. **Tenoxicam-Methylparaben Cocrystal Formation in Aqueous Suspension Formulation**. Poster presented in the AAPS Annual Meeting 2009, poster W4326.

Paper 4

E. Scheubel, L. Adamy and J-M Cardot. **Mycophenolate mofetil: use of simple dissolution technique to assess difference between generic formulations** (2010). Dissolution Technologies *In review*

Poster

E. Scheubel, L. Adamy, ***In vitro*** **dissolution of mycophenolate mofetil: comparison between innovator and generic formulations**- Poster presented at BPS Winter meeting 2008, Abstract 0225 and at the ACCP/ESCP International Congress 2009, Presentation 114E.

1. Introduction

The business environment for the pharmaceutical industry has changed immensely over the past few years. The current blockbuster business model is no longer viable for companies to sustain growth. As the industry faces growing competition from generic drugs, the impact of US healthcare reform in 2010, major price decrease in Europe, the growing threat of biosimilars, the higher demands from regulatory authorities associated with declining product pipelines and rising R&D costs, pharmaceutical executives begin to change the development strategy for NCE. A company can no longer afford to go through the entire drug development process, risking that the drug is rejected by the regulatory agencies, or worse, is withdrawn post-market due to safety concerns e.g. Vioxx, Bextra (Meyer 1992; Vippagunta 2001). Therefore potential issue should be identified and fixed as early as possible.

During the development of new drugs and drug dosage forms the main concerns of the pharmaceutical company is to develop the optimal and constant medicinal product, starting from an Active Pharmaceutical Ingredient (API) which exhibit optimal characteristics up to the production of a robust formulation. This formulation insures a constant Bioavailability (BA) and therapy for the patient over time as independently as possible from the production process. To assist successful oral drug development and post marketed monitoring as well as generic companies in their screening, in vitro dissolution testing has emerged as a preferred method of choice to evaluate development potential of new APIs and drug formulations (figure 1). In the pharmaceutical industry, dissolution may be defined as the amount of drug substance that goes into solution per unit time under standardized conditions of liquid/solid interface, temperature and solvent composition. Dissolution is also the only test that measures in vitro drug release as a function of time. It measures the dynamic effect of static solid state properties. It is a holistic test, and can be considered as a supra indicator of the all phenomena that lead to the release of API into a solution. At the early stage of development, (preformulation), dissolution testing of pure APIs serves as an important tool to evaluate the physicochemical properties of drug candidates and to select the most appropriate solid form for further development. It guides the selection of toxicology and phase 1 formulations for evaluation in animals and humans. When dealing with poorly soluble drugs, observations of potential solubility/dissolution-limited absorption phenomena can strongly facilitate and guide formulation. At later stages of development, dissolution tests are performed with drug products to compare prototype formulations, to elucidate drug release mechanism, as an indicator of stability, the robustness of the manufacturing process, and to assure safe release and reproducibility of the products to the market. Dissolution exhibits clearly a higher predictability if it can be extrapolated directly to in vivo behavior of the medicinal product. This link is called In Vitro In Vivo Correlation (IVIVC) (FDA, 1997; EMEA, 2000) or In Vitro In Vivo Relationship (IVIVR). With the introduction of regulatory guidelines concerning Biopharmaceutics

Classification System (BCS) (FDA, 2000), and IVIVC/R attempts, the dissolution testing can serve as a strong indicator of in vivo performance. Dissolution tests can then be a surrogate measures for bioequivalence (BE), called biowaiver. For high soluble entities, dissolution is a recognized tool to demonstrate equivalence of product before and after certain post approval changes (SUPACs) (FDA, 2000; EMEA, 2002). However several limitations still exist.

Development of a dissolution method may warrant significant and exhaustive evaluation of dissolution profiles in multiple apparatus and media. This effort is rare in discovery and often not fully done in early development phase due to time pressure and few vivo data availability, leading to potential lack of understanding of the effect of the formulation component (API, excipients) properties on manufacturing processes later on after scale up. Prediction of in vivo behavior often requires the use of in vitro dissolution methods reflecting the in vivo GI conditions. Several physiologically based dissolution media, like FaSSIF and FeSSIF (Galia 1998; Jantratid 2008, Klein 2010), have been proposed for this purpose, but their prediction accuracy is still insufficient in many cases. One of the main reasons is the complexity of the physiology of the GI tract (e.g. hydrodynamics) and lack of understanding of the digestion process. In addition, the pharmaceutical industry has been reluctant to make use of the more complex and expensive dissolution media in a routine basis. Furthermore dissolution data quality and purpose may vary depending on its utility and the phase of drug development; these data are sometimes even “sprinkled” in big companies and are then difficult to correlate.

Thus despite their wide use in pharmaceutical development and registration, there is still a lack of thorough understanding of what dissolution could/should measure (API, DP), and the value it adds at various stages of drug development. Even, sometimes industry practices and regulatory expectations with regard to dissolution testing are not similar. The new regulatory Quality by Design (QbD) directives (ICH Q8, Q9, Q10), which encourage pharmaceutical development for in-depth understanding of “causes and consequences”, leads now to a more innovative and science-based approaches in order to improve dissolution method, decrease variability and ensure consistently high quality of dug product.

The present work will focus on the optimization of the existing and alternative dissolution techniques to lay a foundation for QbD principles, IVIVC, and IVIVR. This interplay should serve as a guide for the selection of an appropriate QC or surrogate test(s). Ideally, the final dissolution QC test should monitor the batch-to-batch consistency of the product and, whenever possible, monitor the key biopharmaceutical parameters or Critical Quality Attribute (CQA) of the formulation. However, this goal is frequently not achievable and remains a significant challenge for pharmaceutical formulation and analytical scientist. Examples of this approach are presented in this thesis.

After a description of the current state-of-the-art on dissolution, BCS, IVIVC/IVIVR and relationship with QbD, four aspects of importance of dissolution from early development phases of a new medicine up to generics consideration will be presented in the experimental section. The role and impact of dissolution all along the product life cycle for common solid dosage form will then be discussed with regards to its actual and future use and by taking into consideration the findings of the experimental sections. A decision tree to foster the set up of new dissolution method is proposed. It seems certain that dissolution can be improved as a strong quality control test based on greater understanding of process or release mechanism as well as identifying of CQA.

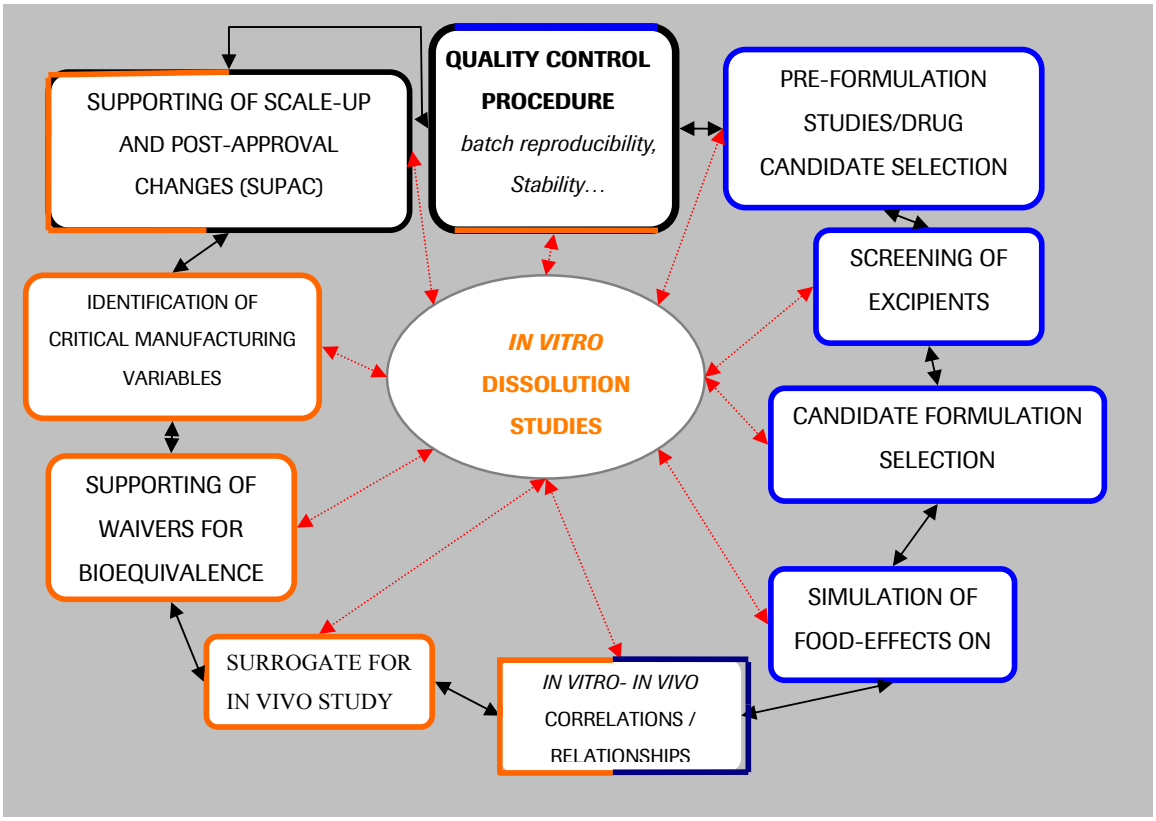


Figure 1: The central role of dissolution testing (early phases of development shaped in blue, late phases in orange, market in black; dotted red arrows show the interplay of dissolution and black arrows show the interaction between the different development phases)

2. State-Of-The-Art

2.1. Dissolution Theory

Dissolution is defined as a dynamic process by which a material is transferred from solid state to solution per unit time. The dissolution of a drug substance can be described in two steps. In the first, molecules are released from the surface to the surrounding dissolution media. This creates a saturated layer, called the stagnant layer, adjacent to the solid surface. Thereafter, the drug diffuses into the bulk of the solvent from regions of high drug concentration to regions of low drug concentration. The theoretical expression most often used to describe the dissolution rate, assuming a sphere, is the Noyes-Whitney equation (Noyes and Whitney, 1897), which was published over one hundred years ago, was adapted by several authors ((Nernst 1904, Brunner 1900 , Underwood 1978) but is still valid.

$$dw/dt = k (Cs – C) \qquad (1)$$

where w is the mass of drug in solution, C is the concentration of drug in solution at time t and Cs is the saturation solubility of the solute (drug) at equilibrium. K is given by

$$k = D.S /h \qquad (2)$$

where D is the diffusion coefficient of the solute (molecular weight and temperature dependent, typically 4-8 x 10-6 cm2 sec-1 (Seki 2003), S is the surface area of the dissolving solid and h the diffusion layer thickness. *k* also known as dissolution rate constant (cm sec-1). It is assumed that in most cases, a rapid equilibrium is achieved at the solid-liquid interface followed by the rate-controlling diffusion across a thin layer of solution, called diffusion layer, into the solution. The latter step is affected by temperature, solution viscosity and composition, degree of agitation, surface, drug particle size and molecular weight. Depending on the particle size, *h* may vary. Under sink conditions, where $C < 0.1Cs$, equation (1) reduces to

$$dw/dt = kCs \qquad (3)$$

Dissolution of drug in a solid dosage form (e.g tablet or capsule) is composed of at least two consecutive steps as well; liberation of solute/drug from the formulation matrix (e.g after disintegration of the tablet resp. deaggregation for IR) followed by dissolution of the drug in the liquid media (according to equation (3)). Thus, in order to achieve dissolution of drug from a dosage form, the cohesive properties of the formulated drug and intrinsic physicochemical properties of the drug molecule play a key role. The overall rate of dissolution will depend on whichever is the slower of these two steps and this should be carefully considered during design of the dissolution method.

In vivo the dissolution rate is influenced by the physicochemical properties of the drug substance, the drug product and additionally by the prevailing physiological conditions in the GI tract (Table 1), which vary between the fasted and fed state as well as within and between subjects.

Table 1: List of the physicochemical and the physiological properties that can influence drug dissolution in the GI tract (Dressman 2000).

Factor	**Physicochemical properties**	**Physiological properties**
Surface area of drug (S)	Particle size, wettability	Surfactants in gastric juice and bile
Diffusion coefficient of the drug (D)	Molecular weight	Viscosity of luminal contents
Stagnant layer thickness (h)		Motility patterns and flow rate
Solubility (Cs)	Hydrophilicity, crystal structure, solubilization	pH, buffer capacity, bile and food composition
Amount of drug already dissolved		Permeability
Volume of solvent available		Secretion, co-administered fluids
Shear force		

Thus the objective of a dissolution testing is to be a discriminatory method that is sensitive to variables that impact the release rate and ideally is predictive of bio-performance. Such variables may include characteristics of the active pharmaceutical ingredient (API) (e.g., particle size, crystal form, bulk density), drug product composition (e.g., drug loading, excipient identity/type and levels), drug product manufacturing process (e.g., compression forces, equipment) at the time of release and during shelf life., and effects of stability storage conditions (e.g., temperature, humidity); Pillay and Fassihi, 1998; Durig and Fassihi, 2000;FDA, 2000) (see also chapter 2.4).

In summary, understanding and control of the release mechanism is therefore a key factor during development of NCE. IR formulation being more impacted by the API properties (see also **paper 1 and 4**) whereas MR formulation mainly by the formulation (see also **paper 2**).

2.2. Dissolution Method

2.2.1. Dissolution Apparatus

A variety of designs of apparatus for dissolution testing have been proposed and tested over the last decades. Different apparatus, procedures and techniques are required for API or different dosage forms because of significant differences in formulation design and the physicochemical properties of the drugs. Dissolution tests have therefore been developed for various drug delivery systems including neat API, immediate release solid dosage forms, several controlled release solid dosage forms and many novel and special dosage forms (see Table 2). Most of the tests with recommended apparatus and other specifications are now available as compendial standards in Pharmacopoeias and are used in pharmaceutical analysis and drug development for the various drug delivery systems (USP 32, PE 6, JP XV). An overview of the most current apparatus is outlined figure 2 and 3. Nowadays the apparatus allow reproducible data and are periodically controlled through mechanical and chemical performance verification tests (USP<711>).

The most commonly used dissolution apparatuses for drug products are the USP Apparatus 1 (basket) and the USP Apparatus 2 (paddle) at 50 to 100 rpm (USP<711>, PE 2.9). Both the paddle and basket methods can accommodate media volumes ranging from 500 to 1000 ml using the standard vessel. For high potent, low dosage drugs the use of 100 ml to 250 ml vessel can be explored (see also **paper 3**) but are not compendial.

USP Apparatus 3 (reciprocating cylinder) and Apparatus 4 (flow-through cell), are used rather earlier in the drug development process and less routinely for QC testing.

USP3 can be used to estimate the drug release profile in the GI tract by using a series of different media in the vessels.

USP4 offers the advantages for instance to overcome the non sink condition in case of low soluble compounds and allows setting the same method for all variants of the manufacturing process (API, galenical intermediate (blend) up to DP) that is of great help during the development and for supporting design space approach (see also chapter 2.5).

By design both USP3 and USP4 allow for a pH change method throughout the test that is of great help by simulation of the GI tract pH and passages.

The intrinsic dissolution tests, i.e. pure drug substance with defined surface area, is traditionally performed with a rotating disk holder (USP <1087>) similar to the one proposed by Wood et al. (Wood 1965). The USP Apparatus 4 is however assumed to have hydrodynamic flow patterns that mimics those found in vivo better than the rotating disk method that can be of great advantage by seeking of In Vitro In Vivo Correlation or Relationship (IVIVC/R) (see also **paper 1** and chapter 2.4). The dissolution of pure API using USP4 is called apparent dissolution (Ph Eur. 2.9).

The amounts of drug substance being limited during the drug discovery phase, miniaturized dissolution testing techniques are highly desired for early development phase. Miniaturized rotating disk apparatuses have therefore been developed (Berger 2007, Persson 2008). Other methods that have been published are mini-scale dissolution tests (Persson 2005, see also **papers 3 and posters**) with computer simulation (Takanao 2008) and channel flow methods (Peltonen 2004, Shah 1975). Other modified system can be found in the literature using for instance Crescent Shaped Spindle (Spagnoli 2006) and can be set up in case where conventional device did not match the desired discriminatory power.

Table 2: Apparatus used for Novel/Special dosage Forms.

AAPS PharmSciTech **2003; 4 (1) Article 7 (http://www.pharmscitech.org).**

Table 1. Apparatus Used for Novel/Special Dosage Forms

Type of Dosage Form	Release Method
Method can be recommended	
Solid oral dosage forms (conventional)	Basket, paddle, reciprocating cylinder, or flow-through cell
Oral suspensions	Paddle
Orally disintegrating tablets	Paddle
Chewable tablets	Basket, paddle, or reciprocating cylinder with glass beads
Transdermals—patches	Paddle over disk
Topicals—semisolids	Franz cell diffusion system
Suppositories	Paddle, modified basket, or dual chamber flow-through cell
More work needed before method can be recommended	
Chewing gum	Special apparatus (PhEur)
Powders and granules	Flow-through cell (powder/granule sample cell)
Microparticulate formulations	Modified flow-through cell
Implants	Modified flow-through cell

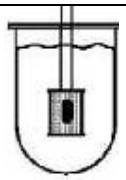
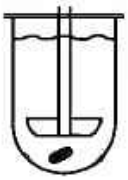
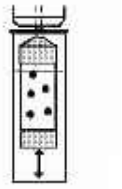
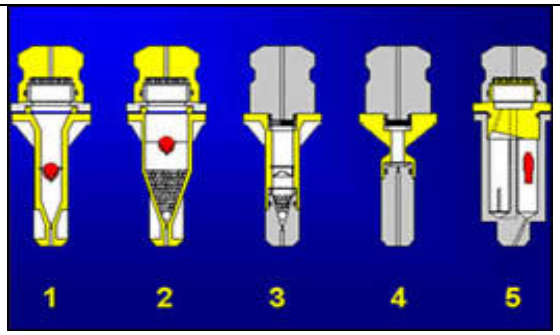
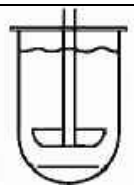
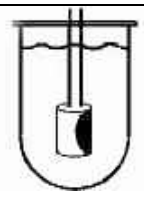
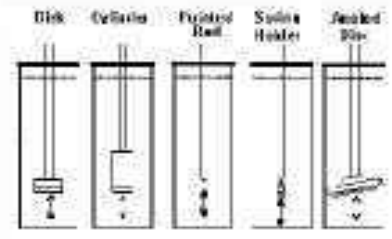
USP Apparatus 1 (Basket) 1 Liter Vessel	
USP Apparatus 2 (Paddle) 1 Liter Vessel	
USP Apparatus 3 (Reciprocating Cylinder) 300 ml Vessel oriented for pH profile, soft gelatine capsule and non-disintegrating multiple units.	
USP Apparatus 4 (Flow through) recommended for water insoluble or sparingly water soluble. Cell for tablets and capsules (1-2) Cell for powders and granulates (3) Cell for implants (4) Cell for suppositories and soft gelatine capsules (5) *((3-4-5) being non USP)*	
USP Apparatus 5 (Paddle over Disc) Trandermal patches using 1 Liter vessel	
USP Apparatus 6 (Rotating Cylinder) Trandermal patches using 1 Liter vessel	
USP Apparatus 7 (Reciprocating Holder) Trandermal patches using 300 ml vessel	

Figure 2: Apparatus Types defined in pharmacopeia for DP

USP <1087> USP<711>, PE 2.9

Figure 3: Apparatus for Intrinsic and apparent dissolution

2.2.2. Dissolution Medium

For batch-to-batch quality testing, selection of the dissolution medium is based, in part, on the solubility data and the dose range of the drug product in order to ensure that sink conditions are met (FIP guideline 1997; FDA 2000; USP <1092>).

When the dissolution test is used to indicate the biopharmaceutical performance of the dosage form, it is important that the proposed test closely simulate the environment in the gastrointestinal (GI) tract than necessarily produce sink conditions for release. Therefore, it is not always possible to develop one dissolution test or select one dissolution medium that ensures batch-to-batch control as well as monitors the biopharmaceutical aspects of the drug product.

The dissolution characteristics of oral formulations should first be evaluated over the physiologic pH range of 1.2 to 6.8 (1.2-7.5 for modified release formulations) in the absence of surfactants since low solubility drugs include those with adequate aqueous solubility at either acidic (e.g., amines) or neutral (e.g., organic acids) pH's. Selection of the most appropriate medium for routine testing is then based on discriminatory capability, ruggedness, stability of the analyte in the test medium, and relevance to in vivo product performance where possible.

For some low solubility compounds, adequate dissolution cannot be obtained with aqueous solutions within the physiologic pH ranges noted previously. For these compounds, an aqueous solution containing a percentage of a surfactant may be used to enhance drug solubility and ensure sink conditions. Surfactants added to the dissolution medium will increase drug solubility significantly. A colloid system, which contains surfactant micelles, will help maintain a poorly water-soluble drug solubilized in an aqueous medium. The dissolution of the drug can be adjusted by changing the concentration of the surfactant in the medium. Sink conditions can be achieved by using higher concentrations of the surfactant. Up to 3% surfactant media are often used in dissolution of poorly water-soluble drugs (Shah 1995). However, the human GI track does not have

such a high concentration of surfactant, therefore it is not a surprise to find out that the dissolution results obtained from media of high surfactant concentrations have poor correlation with bioavailability. A biorelevant medium will need a similar surface activity as bio-fluids. The need for surfactants and the concentrations used should be justified. Standard ionic or non-ionic surfactants are sodium lauryl sulfate (SLS), Tween, CTAB, Cremophor, HTAB, Triton, Terigitol, Cyclodextrins and Lecithin. In general, non-ionic detergents (e.g., Tween) are considered more biologically relevant, and thus are often the preferred first choice when considering the addition of a surfactant (it is to note that tween is the only defined surfactant in the Japan Pharmacopeia). Surfactants can be used as either a wetting agent or, when the critical micelle concentration (CMC) is reached, to solubilize the drug substance.

For some water-soluble drugs, pH of the dissolution medium has less effect on dissolution, but surfactants added to the dissolution medium will increase drug solubility significantly. With this regard, a USP4 method using open system (see previous chapter) can be an alternative to a classical method using surfactant.

To simulate the in vivo conditions more nearly than just approximating the pH and volume, substances that occur in the GI fluids can be added to the media. Several media simulating the GI fluids have been proposed, including gastric (e.g. FaSSGF) and intestinal fluid (e.g. FaSSIF and FeSSIF) (Galia 1998; Sunesen 2005) and efforts to further improve them are ongoing (Fujioka 2007, Jantrid 2008, Lue 2008, Ghazal 2009, Klein 2010). So called, biorelevant media, which are designed to closely simulate physiological secretions, aim to better link in vitro with in vivo performance. Dressman et al. (Dressmann 1998, 2000) created dissolution media through consideration of the main factors which are generally expected to influence dissolution in vivo. Values of lecithin, taurocholic acid, osmolality, surface tension, buffer capacity and pH were adjusted to physiological values.

Even milk can be used during drug development to approximate condition in the postprandial stomach. Milk contains similar ratios of protein/fat/carbohydrate to that found in typical Western diet. Mechanisms by which milk can improve drug solubility include solubilisation of the drug in the fatty part of the fluid, solubilisation in the caseine micelles and, for weak acids, the favorably high pH values. This media presents however some difficulties in filtering and separating the drug from the medium, making them unsuitable for routine QC.

To reflect differences between the fasted and fed state, different media were established: FaSSIF (Fasted State Simulating Intestinal Fluid), FeSSIF (Fed State Simulating Intestinal Fluid). Usually taurocholic acid and phosphatidylcholine from egg are used as bile salts and lecithin components, respectively. To represent triglycerides and fatty acids glycerol monooleate and sodium oleate are commonly used. Osmolality is adjusted to physiological values with NaCl. But even though the media simulate most relevant characteristics, such as concentration of solubilising substances,

buffer capacity, pH and solubilisation capacity of drugs, they are not a one-to-one copy of gastric or duodenal juice. For example, pH and buffer capacity vary considerably as a function of digestion and as a function of location within the intestine. FaSSIF is based on a non-physiological buffer system, phosphate buffer (0.029 M, pH 6.5) whereas FeSSIF are based on a partly physiological acetate buffer (0.144 M, pH 5.0). Additionally, compared to many surfactants with micelle aggregation numbers >60 (Balakrishnan 2004) bile salt structures give values <10 (Sun 2003) and hence bile salts are expected to solubilize less compound. Lecithin added to bile salts (mixed micelles) leads to an improvement of the solubility and of the dissolution rate of some low solubility drugs (Naylor 1995). However, the effective diffusivity of mixed micelles is approx 100-fold lower than in comparable taurocholate solutions since lecithin also increases the micellar diameter (Naylor 1995). The increase depending on the dilution (Sugano 2007). Furthermore, the buffer capacity of previously used FaSSIF and FeSSIF media (Gallia 1996 1998, Dresssman 2000) were too high compared to values measured in human aspirates (Kalantzi 2006) and hence self-buffering effects may have been underestimated with these media. Recently, this was corrected by the introducing new media with lower buffer capacity (Jantrid, 2008, see Table 3). It is to note that, addition of SDS can interfere with the vesicular drug solubilizing system of the biorelevant medium (e.g. FaSSIF) and antagonized its solubilization capacity (Buch 2010).

However, the conventional preparation method of these fluids shows some disadvantages, it is time-consuming, requires organic solvents, requires daily preparation and sometimes does not result in a usable medium. Recently commercial “ready to use” preparations are available and can perhaps help to better standardize and simplify the realization of the test (SIF Powder from ePhares.com) (Kloefer 2010).

The fact that very different combinations of properties between the media were shown to produce similar dissolution enhancement levels in FaSSIF and drug-specific concentrations of SLS or Tween 80 underlines the importance of the evaluation of surfactant-facilitated dissolution as a whole. Although it is obvious due to the specificity of the drug-surfactant interactions that the in vivo prognostic amounts of synthetic surfactants have to be adjusted drug-specifically, some studies shows the great potential of substituting FaSSIF with simple and cost-effective conventional surfactant media (Zoeler 2007) (see also **paper 1, paper 2** and **paper 4**).

Table 3: Composition of FaSSIF and FeSSIF according to Jantratid et al. (Jantratid 2008).

	FaSSIF	FeSSIF
Bile salt (sodium taurocholate) (mM)	3	10
Phospholipid (lecithin) (mM)	0.2	2
Maleic acid (mM)	19.12	55.02
Sodium hydroxide (mM)	34.8	81.65
Sodium chloride (mM)	68.62	125.5
Glyceryl monooleate (mM)		5
Sodium oleate (mM)		0.8
pH	6.5	5.8
Osmolality (mOsm kg^{-1})	180 ± 10	390 ± 10
Buffer capacity (mmol l^{-1} ΔpH^{-1})	10	25

2.2.3. Qualification of Apparatus

Ensuring the quality of data generated by analytical equipment includes an overall approach to equipment quality. Pharmaceutical scientists have long accepted that equipment qualification—installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ)—under good manufacturing practices (GMPs) should be executed to verify acceptable quality of output from multi-component analytical instrumentation.

The dissolution test system consists of the mechanical apparatus (itself composed of several components), the physical environment in which the apparatus exists, the analytical procedure, and the analyst.

The suitability of the apparatus for the dissolution testing depends on both the physical and chemical qualification which qualify the equipment for further analysis. Besides the geometrical and dimensional accuracy and precision, as described in harmonized Pharmacopeia (USP, EP, JP), any irregularities such as vibration or undesired agitation by mechanical imperfection are to be avoided. Temperature of the test medium, rotation speed/flow rate, volume sampling probes and procedures need to be monitored periodically.

For instance, USP endorses the concept of mechanical calibration (grounded in metrology), conducted at periodic intervals, to ensure that the mechanical components meet specifications and are in a state of control. These mechanical checks, however, are necessary but not sufficient because they ignore the chemical and kinetic aspects of the dissolution procedure, an oversight that is common in most chemical tests. One vital aspect of qualification is therefore the Performance Verification Test (PVT) (USP 2010). The use of PVT tablets (for apparatus 1 and 2) is the only standardized approach for conducting dissolution tests and has been able to identify variability or operator failures (Gray 2002, USP 2010). Suitability tests have also been developed for Apparatus 3, using specific calibrators. The aim is to generate a set of calibrators for each and every compendia dissolution test apparatus (Hanson 2004, Dressman and Krämer 2005)

2.2.4. Analytical methods associated with the Dissolutions

The universal analytical separation method with acceptable selectivity and sensitivity is high-performance liquid chromatography (HPLC), with transfer to the more efficient ultra-performance liquid chromatography (e.g. UPLC (Waters) or RR LC (Agilents)). HPLC is often the method of choice even though it is less time efficient than UV/VIS due to the fact that during early phase development multiple formulations and strengths are screened and potential interferences from the formulation matrix or medium or even degradation of the active can be separated easily by HPLC. Further, large variations in sample concentration can often be dealt with simply by adjusting injection volume. Later on UV/VIS can be re-evaluated for QC routine and efficacy increase when the formulation, the strength and the stability is well known. For QC and release purpose the analytical methods need to be validated with regard to selectivity, linearity and accuracy. (<USP<1092>, Gray 2009).

Spectroscopic in situ methods with fiber optics can also be used to analyze the liquid phase in dissolution testing (Josefson 1988, Cho 1995) and, if desired, in situ solid phase analysis can be performed by XRPD (Debnath, 2003) or Raman spectroscopy (Aaltonen 2006). Alternative detectors to UV/VIS can be used if the analyte contains poor chromophores or low absorbance response. Some of these are: evaporative light-scattering detector (ELSD), electrochemical detector (ECD), refractive index detector (RI) or mass spectrometry (MS).

Additionally qualitative imaging technologies have been developed recently (Malaterre 2008), using for instance Magnetic resonance imaging (MRI), tomography (Zeitler 2008) or NMR imaging (Djemai 2008), EIT, Electrical Impedance Tomography, (Rimpiläinena 2010).

A newly example using XRay topograph is presented in this work as illustration of these approach (see **paper 2 supplement**). These techniques allow a better understanding or visualization of the intrinsic release mechanism and clearly participate to a sound rational for the development of new robust medicine, that is in line with the Quality by Design concept (see chapter 2.5) and highlight some future broaden potentials for dissolution method.

2.2.5. Discriminating Power of a Method

The discriminatory power of the dissolution method depends on the method's ability to detect changes in the drug product performance. Ideally, the dissolution test conditions should discriminate product changes that may affect biopharmaceutical product performance. However, unless an IVIVR or IVIVC exists (see chapter 2.4) for the product, variations in dissolution behavior may or may not reflect variations in the product's in vivo performance.

To determine if a dissolution method can discriminate the impact of product changes, the method needs to be challenged. The most common way to challenge the discriminatory power of the

method is to test formulations manufactured with differences resulting from changes in the characteristics of the API (e.g., particle size, crystal form, bulk density), drug product composition (e.g., drug loading, excipients identity/type and levels), drug product manufacturing process (e.g., dosage form, equipment variables as under or over granulation), and effects of aging (e.g., temperature, humidity).

These experiments should be designed on a case-by-case basis , based on a DoE, in consultation with the galenist, chemist and analytical specialist. At this stage the collaboration between the expertises is clearly a key factor. Thus, the change in the drug product can be evaluated versus the change in the dissolution data. If the data show a measurable difference for the key variables, then the method may be considered a discriminating test for critical manufacturing variables. Any differences in the dissolution rates as a result of the selected variables may or may not have impact on the in vivo product performance.

The dissolution method tends to evolve depending on its utility for drug development and should be re-evaluated and optimized (if needed) when human bioavailability data are available from the clinical formulations. During further method development, optimization, and before selection of the final method, the formulations utilized in the late phase clinical studies are tested using various medium compositions (e.g., pH, ionic strength, surfactant composition). The effect of hydrodynamics on the formulations should also be evaluated by varying the apparatus agitation speed. If a non-bioequivalent batch is discovered during a bioequivalency study, the dissolution methodology should be further modified to allow differentiation of non-bioequivalent batches from the bioequivalent batches by dissolution specification limits, if possible. This would ensure batch-to-batch consistency within a range that guarantees comparable biopharmaceutical performance in vivo. Once a discriminating method is developed, the same method should be used to release product batches for future studies, if possible. The biorelevant method may not always be feasible, and may or may not be the same as the QC method due to the scope and limitations of such a method.

2.3. Biorelevance of Dissolution Testing

2.3.1. BCS Definition

To be efficacious, the active drug substance must be released from the drug product and absorbed into the systemic circulation so that it can be transported to its site of activity. The overall efficiency of this process contributes to the bioavailability of the drug substance and involves two steps, dissolution and absorption, or permeability, as defined within Food and Drug Administration (FDA) guidelines concerned within the Biopharmaceutics Classification System (BCS).

The BCS was first described in 1995 (Amidon, 1995) and its principles have been used in several FDA guidances (FDA, 1995, 1997, 2000). The BCS is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. The main parameters for influencing rate and extent of absorption of a drug substance through gastrointestinal membranes and having significant influence on its bioavailability. When combined with the dissolution of the drug product, the BCS takes into account three major factors that govern the rate and extent of drug absorption from immediate release solid oral dosage forms:

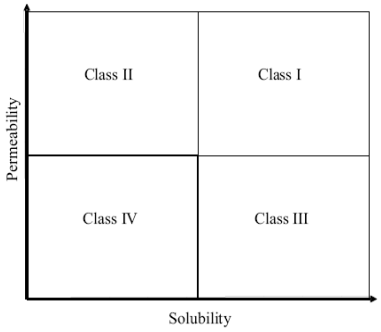
- Solubility
- Intestinal permeability

Low solubility compounds, based on the BCS, are defined as compounds whose highest therapeutic dose is not soluble in 250 mL or less of aqueous media from pH 1.2 to 7.5 at 37ºC. The highest dosage form divided by the lowest solubility in the pH range 1.2 to 7.5 should be less than 250. It's important to note that solubility is mostly a property of the API and its salt form. Kinetic solubility is usually determined by measuring the concentration of a saturated solution after equilibration at 37ºC usually for 1 hr to 24 hrs. The equilibration time depends on the test duration time as well as the physical and chemical stability (e.g., conversion of salt to free base in vitro) of the drug.

High permeability is defined as human absorption of 90% or more of the administered dose (FDA 2000). Rapidly dissolving is defined as no less than 85% of label claim dissolved within 30 minutes for either USP Apparatus I at 100 rpm or USP Apparatus II at 50 rpm in pH 1.2 (0.1 N HCl or simulated gastric fluid USP without enzyme), pH 4.5 buffer and pH 6.8 buffer (or simulated intestinal fluid USP). Aside from API solubility, dissolution rate is a function of the product disintegration (porosity, lubrication, granules, etc). This put then one important thing in evidence, the classical BCS concept does not integrate dissolution rate.

Low solubility, high permeability compounds are classified as Class II compounds. For these compounds which have increased potential to demonstrate intrinsic dissolution-limited absorption (rate of drug solubilization is much less than the rate of drug absorption) an In Vitro In Vivo Relationship (IVIVR) or In Vitro In Vivo Correlation IVIVC may be possible (Lipka 1999) (see chapter 2.4). Low solubility, low permeability compounds are classified as Class IV compounds and may have solubility and permeability limited absorption. High solubility, high permeability compounds are classified as Class I compounds and high solubility, low permeability compounds are classified as Class III compounds. See Table 4

Table 4: Biopharmaceutical Classification System (BCS)

Class I:	High solubility,	high permeability	
Class II:	Low solubility,	high permeability	
Class III	High solubility	low permeability	
Class IV:	Low solubility	low permeability.	

The BCS characteristics (solubility and permeability), together with the dissolution of the drug from the dosage form, takes the major factors that govern the rate and extent of drug absorption from dosage forms into account (Charkoftaki 2010).

2.3.2. Extension of BCS

In 2005, Wu and Benet proposed an extended BCS, the Biopharmaceutics Drug Disposition Classification System (BDDCS), which introduced first pass metabolism in the intestine and/or liver as additional factor for the categorization of drugs (Wu 2005, Benet 2009).

As well, Bergstrom *et al.* (Bergstrom 2003) proposed a modified Biopharmaceutical Classification System, in which they categorized the drugs into six classes based on the solubility and permeability. The solubility was classified as "high" or "low" and the permeability was allotted as "low", "intermediate," or "high". This new classification was developed based on the calculated surface area descriptors on the one hand and solubility and permeability on the other. Surface areas related to the nonpolar part of the molecule resulted in good predictions of permeability. It was tentatively concluded that these models would be useful for early indication with regard to the absorption profiles of the compound during the early stages of drug discovery so that the necessary modifications can be made to optimize the pharmacokinetic parameters

BCS and BDDCS are useful tools in discovery and early development to identify rate limiting steps, to rank order compounds, and to communicate potential higher formulation risks. In later phases, the BCS is used as a regulatory tool to identify IVIVC and to obtain waivers for in vivo BA and BE testing according to SUPAC (FDA 1995), using dissolution testing at different pH as main method to show similarity.

Waivers are designed to replace in vivo BE studies for immediate release products by in vitro dissolution which can both reduce costs and improve the quality of medicines. Waivers were originally designed only for class 1 drugs (FDA guidance, 2000; EMEA, 2002). In that case, the application may be based on in vitro dissolution and permeability data together with scientific justification of linear pharmacokinetics within the dosing range, a proof that the drug does not have a narrow therapeutic index and that the excipients do not have pharmacokinetic interactions with the drug. Recently, EMEA (EMEA 2010) allows biowaivers for BCS Class III drugs in specific cases. This is different to the FDA Guidelines, where the BCS approach is only applicable to BCS Class 1 drugs. This class has been proposed in many publications as appropriate for biowaivers if the effects of excipients on the gastrointestinal transit time and permeability can be excluded (Blume, Schug 1999; Yu et al. 2002; Cheng et al. 2004; Vogelpoel et al. 2004; Jantratid et al., 2006). BCS III drugs have low permeability, which, rather than dissolution, is the limiting factor for absorption. This was clearly seen in a bioavailability study of cimetidine immediate-release and controlled-release formulations (Jantratid 2006). Permeability was the rate-limiting step for in vivo absorption even when more than 85% of the drug dissolved in 90 minutes. In many publications, BCS III drugs have been suggested for biowaivers if more than 85% of the drug dissolves in 15 minutes (Yu 2002; Polli 2004; WHO, 2006, Fagerholm 2007).

The actual biowaiver strategies are highlighted in figure 12 and also in chapter 2.5.

2.3.3. Application of BCS in the formulation development

Once the solubility and permeability characteristics of a drug are known, the formulation scientist can then, based either on BCS or BDDCS, easily decide which drug delivery technology will best help in getting the optimum pharmacokinetic characteristics.

The major challenge in the development of drug delivery systems for a class I drug is to achieve a targeted release profile associated with the particular pharmacokinetic and pharmacodynamic properties. Formulation approaches include both the control of release rate and physiochemical properties of drugs like the pH-solubility profile of the drug. Dissolution should be kept as simple as possible and whenever possible rapidly dissolving should be reached for IR.

The formulation systems that are developed for class II drugs are mostly based on the micronization, lyophilization, addition of a surfactants, formulation as emulsions and micro

emulsion systems, use of complexing agents like cyclodextrins, and so on. Dissolution often needs to be performed with addition of surfactant.

Class III drugs are required for technologies that address the fundamental limitations of absolute or regional permeability. Dissolution is similar to BCS class 1.

The class IV drugs present a major challenge for the development of drug delivery systems and the route of choice, due to their poor solubility and permeability characteristics. These are often administered by parenteral route with the formulation containing solubility enhancers.

2.4.IVIVC/R

For a dissolution test to be valuable in linking the formulation with efficacy and performance characteristics, establishment of IVIVC or IVIVR is crucial. The IVIVC or IVIVR dissolution method can then serve as a guide for the development of a meaningful quality control method, which will occur in the different clinical development phases.

2.4.1. Definitions

Definitions of *in vitro-in vivo* correlations were proposed by the FIP (International Federation of Pharmaceutics), the USP (United State Pharmacopeia), the FDA (Food and Drug Administration (US)), the EMEA (European Medicinal Evaluation Agency (Europe)) and ICH (International Conference for Harmonisation). All those definitions are hopefully globally similar. For the USP (chapter <1088>) the definition is as follows:

"*The term in vitro-in vivo correlation first appeared in pharmaceutical literature as a result of the awareness of the concepts of bioavailability and of in vitro dissolution rate determinations. The term in vitro-in vivo correlation refers to the establishment of a rational relationship between a biological property, or a parameter derived from a biological property produced by a dosage form, and a physicochemical property or characteristic of the same dosage form. The biological properties most commonly used are one or more pharmacokinetic parameters, such as Cmax or AUC, obtained following the administration of the dosage form. The physicochemical property most commonly used is a dosage form's in vitro dissolution behaviour (e.g., percent of drug released under a given set of conditions). The relationship between the two properties, biological and physicochemical, is then established quantitatively.*

With the proliferation of modified-release products, it becomes necessary to examine the concept of in vitro—in vivo correlation in greater depth. Unlike immediate-release dosage forms, modified release products cannot be characterized using a single-time point dissolution test. Furthermore, with a modified-release product a patient is to show a specific plasma level curve covering a finite time period, usually 12 to 24 hours. There must be some in vitro means of assuring that each batch of the same product will perform similarly in vivo. An in vitro-in vivo correlation would satisfy this

need. Initially it was thought that developing a meaningful correlation for immediate-release dosage forms would be an easier task than for modifled-release products. However, because of the nature of the principles upon which each type is based, it is believed that an in vitro-in vivo correlation is more readily defined for modified-release dosage forms."

For FDA in the "Guidance for Industry Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations", IVIVC are defined in the glossary as "*In vitro/in vivo correlation: A predictive mathematical model describing the relationship between an in vitro property of an extended release dosage form (usually the rate or extent of drug dissolution or release) and a relevant in vivo response, e.g., plasma drug concentration or amount of drug absorbed*."

The European Agency as well as ICH use the term in various notes for guidance without giving an exact definition (ICH Q8 and EMEA Note for guidance on the quality of modify released product section I, Note for guidance on product development, Note for guidance on BE/BA).

All those definitions could be summarized as relationships (*in vitro in vivo relationship IVIVR*) or correlations (*in vitro in vivo correlation IVIVC)* observed between parameters or curves derived from *in vitro (dissolution)* and *in vivo (bioavailability or bioequivalence)* studies, irrespective of the mathematical definition of the terms. IVIVR or IVIVC try to establish a link either between the full dissolution curves and the in vivo absorption curves or between some parameters derived from in vitro curves (for example time to have x% dissolved or % dissolved at certain times) and in vivo curves (mainly bioequivalence parameters: Cmax-AUC). IVIVR and IVIVC are two ways to establish a link between in vitro and in vivo data.
It is not easy to differentiate in vitro in vivo relationships from in vitro in vivo correlations.
The terms relationship and correlation are close together. Merriam Webster dictionary defines "*a relationship as the state to be related or interrelated*" and a correlation as "*a relation existing between phenomena or things or between mathematical or statistical variables which tend to vary, be associated, or occur together in a way not expected on the basis of chance alone*". From a statistical point of view, a correlation could be established when both variables are normally distributed and randomly obtained and a relationship when only one of the two is randomly obtained. From a biopharmaceutical point of view the two terms are often define as:

- IVIVC: a "quantitative" linear mathematical model relating in vitro property of a dosage form (usually dissolution or release) and a relevant in vivo response (usually "absorption" curve or parameters derived from plasma time-concentration curve). The IVIVC could be used (in case of level A) as a biowaiver for in vivo BA studies.

- IVIVR: a “qualitative” ranking between in vitro and in vivo data that indicates qualitative tendencies. This IVIVR helps in the identification of key factors.

There are four levels of IVIVC that have been described in the FDA guidance, which include levels A, B, C, and multiple C.

Level A Correlation: This correlation represents a point-to-point relationship between in vitro dissolution and in vivo dissolution (input/absorption rate). Level A IVIVC is also viewed as a predictive model for the relationship between the entire in vitro release time course and entire in vivo response time course. In general, correlations are linear at this level. Although a concern of acceptable non-linear correlation has been addressed, no formal guidance on the non-linear IVIVC has been established. Level A correlation is the most informative and very useful from a regulatory perspective.

Level B Correlation: In Level B correlation, the mean in vivo dissolution or mean residence time is compared to the mean in vitro dissolution time by using statistical moment analytical methods. This type of correlation uses all of the in vitro and in vivo data; thus, it is not considered as a point-to-point correlation. This is of limited interest and use because more than one kind of plasma curve produces similar mean residence time.

Level C Correlation: This correlation describes a relationship between the amount of drug dissolved (eg, % dissolved at 1 hour) at one time point and one pharmacokinetic parameter (eg, either AUC or Cmax). Level C correlation is considered the lowest correlation level as it does not reflect the complete shape of the plasma concentration time curve. Similarly, a multiple Level C correlation relates one or more pharmacokinetic parameters to the percent drug dissolved at several time points of the dissolution profile and thus may be more useful. Level B and C correlations can be useful in early formulation development, including selecting the appropriate excipients, to optimize manufacturing processes, for quality control purposes, and to characterize the release patterns of newly formulated immediate-release and modified-release products relative to the reference.

Anyway those definitions like the mathematical one do not imply a causal relationship. Two parameters could be related together by a direct cause-effect relation or by an indirect relation. The figure 4 shows that the dissolution curve reflects numerous underlined phenomena. A difference in dissolution could reflect, as expected in IVIVC, a difference in release from the drug dosage form

but also a difference in API characteristics which is of low interest for IVIVC, since it can be addressed by other analytical method.

The causality will be assessed during the validation process (called predictability) taking into account the API characteristics and formulation process in an ideal case.

Establishment of IVIVR or IVIVC could be summarized as "finding a correlation or relationship between in vivo and in vitro results". In vitro, the dissolution reflects a number of factors (cf figure 4 & 5& 6). For example a slow dissolution reflects either a slow dissolution rate or low solubility of the API or a slow release from the drug dosage form (figure 7).

The API characteristics like dissolution rate or solubility belong to the chemistry field and not to the pharmaceutical development field and could be considered to be perfectly studied and defined before the development of the pharmaceutical formulation: normal or micronized API, acid or salt, etc…. The main parameters studied in vitro must reflect the release of the drug from the drug dosage form (figure 7) and not the API characteristics.

It is useful to have a full characterisation of the various batches of API, included in the formulation and the manufacturing process of the final formulation so as to be able to establish that any differences observed in vitro will be linked with the formulation and not with a problem of API sourcing or manufacturing. In this case the manufacturer can predict the in vivo behaviour of the formulation form of its composition without the influence of the API sourcing or manufacturing.

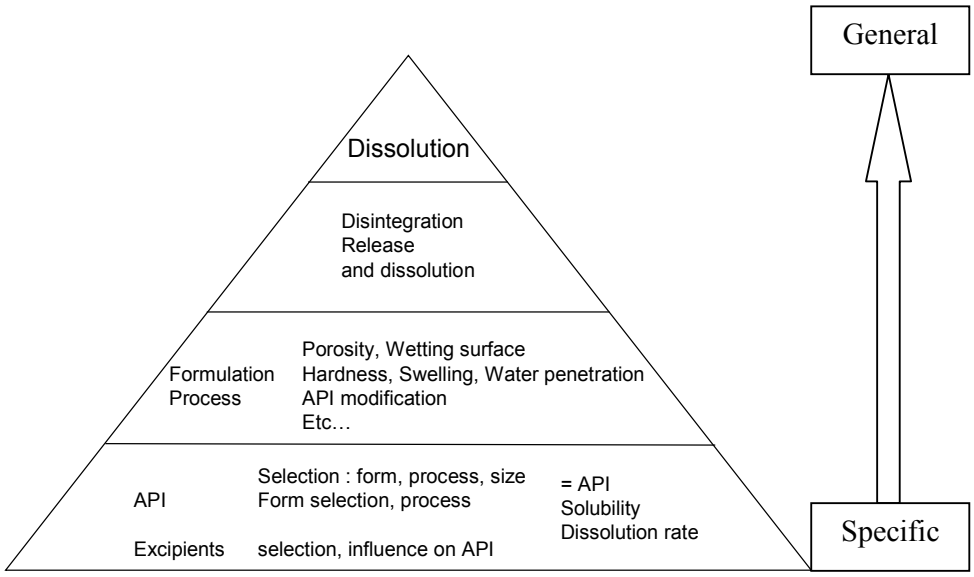


Figure 4: Dissolution as the reflect of various phenomena (Adapted from A Quality by Design Approach to Dissolution Based on the Biopharmaceutical Classification System, R. Reed)

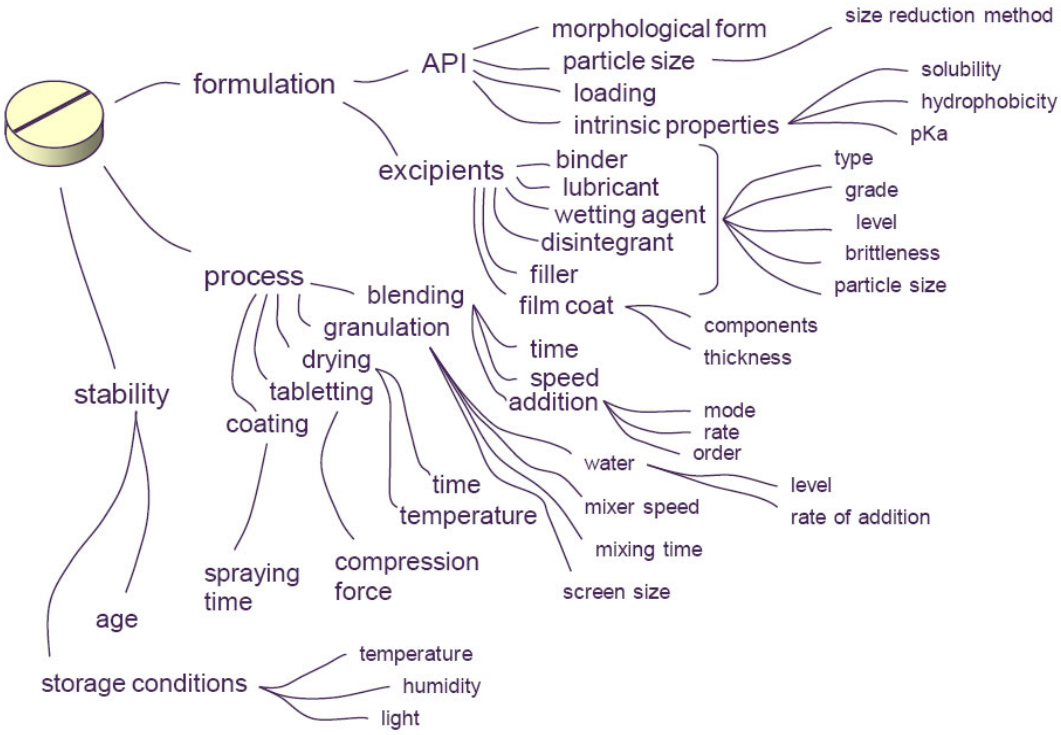


Figure 5: Factors affecting the in vitro dissolution (From Dr Dónal Murphy, Astra Zeneca UK, IRR Conference, May 07, Budapest)

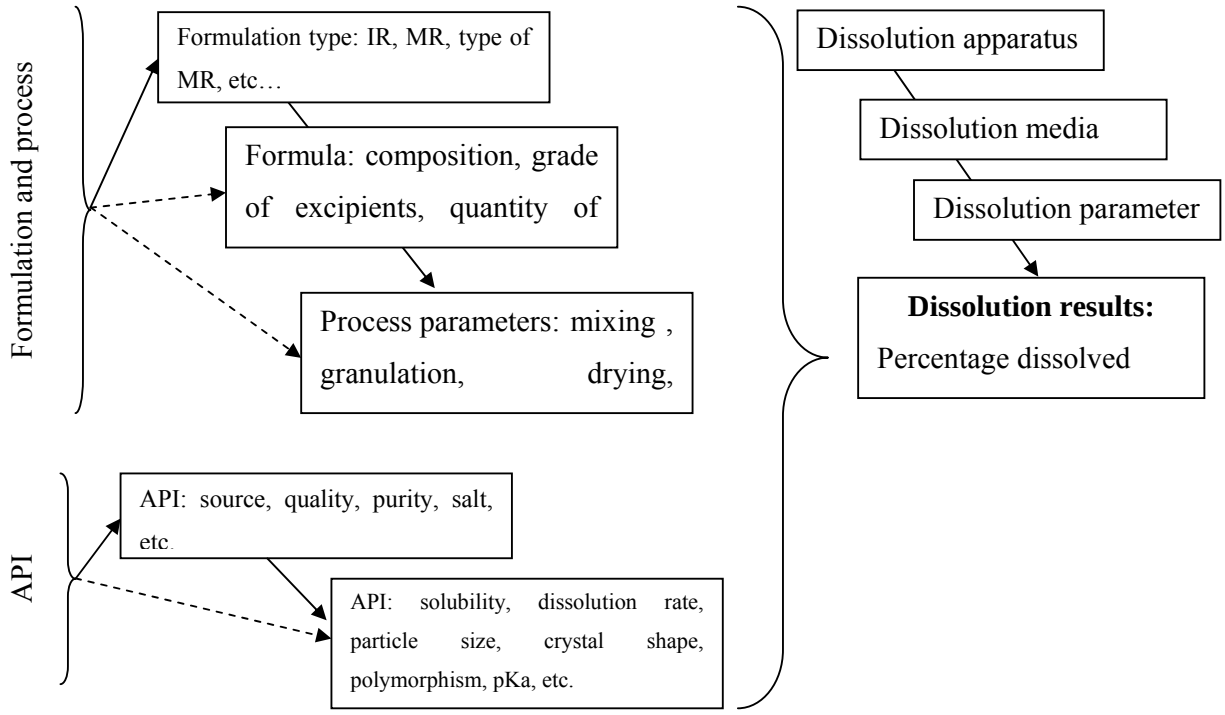


Figure 6: Dissolution as a reflection of the pharmaceutical complexity of the product, continuous line: directly accessible information, dashed line: underlined properties of material.

The figure 7 summarizes the behaviour of a drug administered per oral solution (os), the observed plasma concentration reflects the slowest of the three phenomena: the dissolution rate of the API; the release from the drug delivery form (DDF) in vivo and the absorption from the GIT. A part of the information could be given by the BCS and the other metrics derived from it (absorption, dose and dissolution numbers).

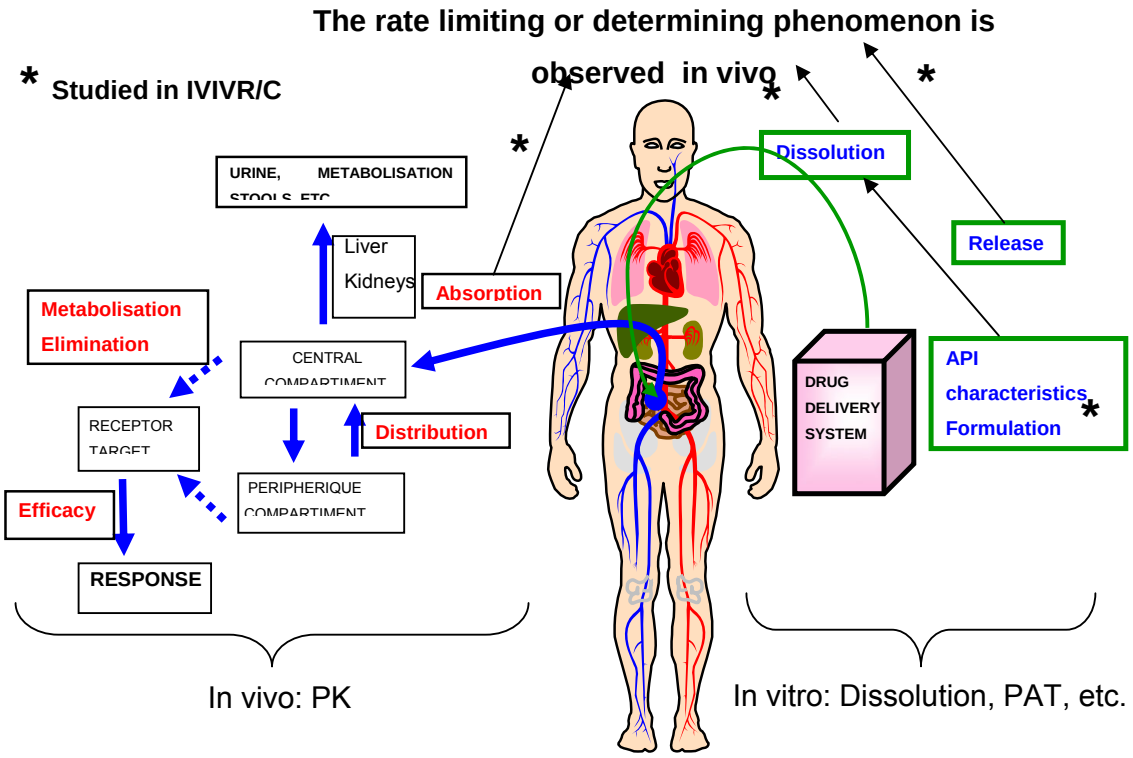


Figure 7: Plasma concentration curve after the administration of a per os formulation (adapted from Cardot, Sotax Workshop Basel 2010)

The plasma concentration curve is a global representation; it depends on drug input in the blood flow which depends on the dosage form (release rate), the properties of the API (solubility, dissolution rate, permeability, type of absorption, efflux, etc.), and thereafter its pharmacokinetics input processes. The disposition of the drug after input depends only on the drug and patient (figure 8).

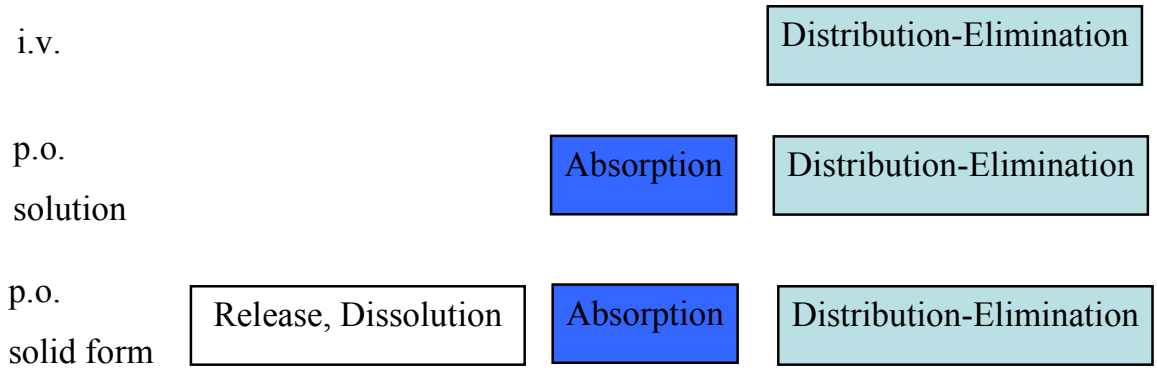


Figure 8: main phenomena after administration of various formulations (FDA 1997))

Two different formulations could exhibit different blood profiles because either the release of drug at site of absorption was not completed (formulation problem), or the drug in solution at site of absorption is insufficient (API characteristics) or due to a non linear or inconstant physiological

process (high or saturable first pass metabolism, low GI permeability, presence of efflux, non passive absorption). When the synthesis of the API is stabilized, the release of the drug from the DDF is the only factor which can have a role and can be studied in vitro. Figure 9 sums up those phenomena.

If in vivo, absorption is the slowest of the three processes (kd>kp in figure 9), then absorption is the limiting factor for the behaviour of the drug in the blood is then limited by this absorption in vivo. The release or dissolution rate of the drug is then of no importance.
If slowest phenomena in vivo between dissolution release and absorption is the release (kd<kp in figure 9 the behaviour of the drug in the blood is then limited by this dissolution/release in vivo. The drug concentration observed in blood will then depend not of the absorption of the drug but of its dissolution/release. This dissolution release could be easily studied in vitro. The aim of IVIVx is to relate the observed release dissolution in vivo considered as the limiting factor to the in vitro observed dissolution. In this last proposition 2 cases existed. If the API dissolution is slower than the release (ks<kr) then the observed IVIVx will be between an API characteristics (like crystal size, intrinsic solubility, etc., usually not investigated by dissolution tests) and the blood input. If the API dissolution is faster than the release (ks>kr) then the observed IVIVx will be between the drug release (which is normally studied by a dissolution test) and the blood input.

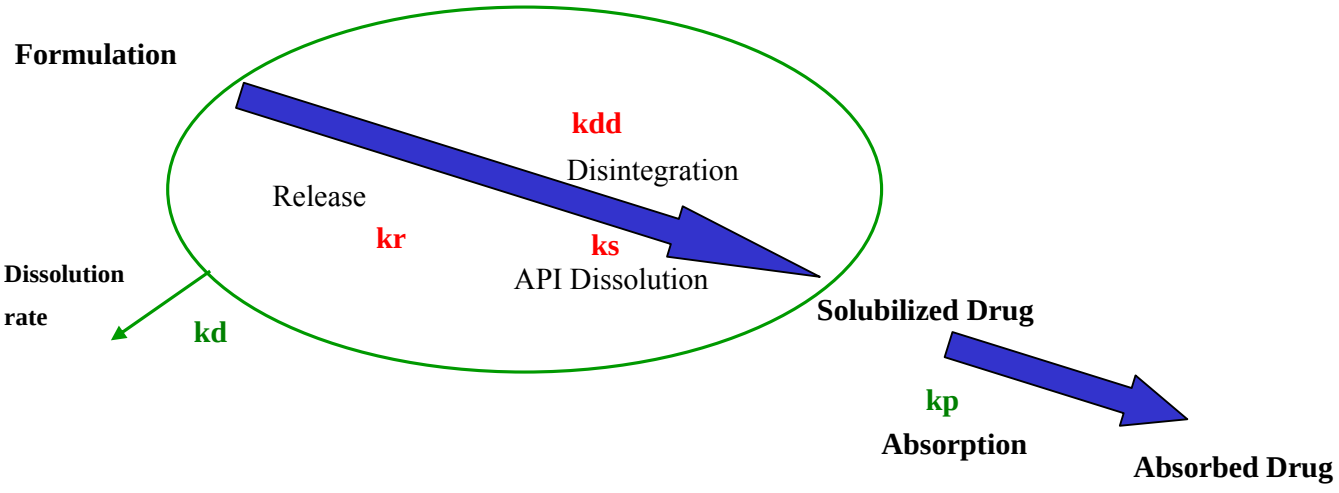


Figure 9: Representation of the main phenomena leading to absorption in vivo, in green apparent observed (macro) phenomena, in red underlined dissolution (micro) phenomena

The aim of IVIVC is to study the relation between the formulation and process parameters described in figure -1 usually studied by a dissolution test and the in vivo input. The main

parameters are summarized as kd and kp in figure 9, kd dependent on 3 main components and it is assumed for IVIVC that ks is not the limiting factor).

IVIVC and mainly level A IVIVC can be used in numerous cases. It is a powerful tool for development; it guarantees a gain of time and the in vivo performances. It allows determining the release characteristic over time. It can be used as a process control and quality control for the validation of the manufacturing control limits (dissolution limits). It facilitates certain regulatory determinations like minor variations and can be used as a surrogate of certain bioequivalence studies for extended release formulations (bio waiver) like for example in case of small modification in the quantitative composition of the release excipients, of the dose strength, of small modification of the manufacturing process or modification of the production site

In ICH Q8, regarding development of formulations, a full paragraph is on the use of IVIVC to compare clinical formulation to final market image, this text emphases the interest of IVIVC and the possible use of IVIVC to set dissolution limits: "*Information from comparative in vitro studies (e.g., dissolution) or comparative in vivo studies (e.g., bioequivalence) that links clinical formulations to the proposed commercial formulation described in 3.2.P.1 should be summarized and a cross-reference to the studies (with study numbers) should be provided. Where attempts have been made to establish an in vitro/in vivo correlation, the results of those studies, and a cross-reference to the studies (with study numbers), should be provided in this section. A successful correlation can assist in the selection of appropriate dissolution acceptance criteria, and can potentially reduce the need for further bioequivalence studies following changes to the product or its manufacturing process.*"

They point out also the fact that "*… an establish IVIVC may reduce the number of bioequivalence studies during product development, be helpful in setting specifications and be used to facilitate certain regulatory decicion (e.g. scale-up and post approval variations)*" and it is stressed out that IVIVC should always be considered as a possible way of developing a formulation by the applicant. Even if a correlation is used for developing a drug and mainly for generic some limitations exist "…*correlation is not intended to serve as a basis for claiming bioequivalence between different product from different MA applicants, based on in vitro data only*" that being normal as the IVIVC established on a formulation using a release mechanism could not be used for another release mechanism and due also to the fact that all the key factors of the production are not know for the reference formulation.

In development IVIVC is a tool with allow speed up development as it can be used to understand better the in vivo release mechanism, to establish the key factors of the formulation and to de risk bio equivalence studies. IVIVC may reduce the number of in vivo studies during the development and can be established using forecasted studies with no additional cost.

A biowaiver is the use of in vitro dissolution curve in place of bioequivalence study to prove equivalence of formulation or process. That is based on the simulation (calculation) of the plasma-concentration curves based on the dissolution profile and on the established IVIVC (Figure 10)

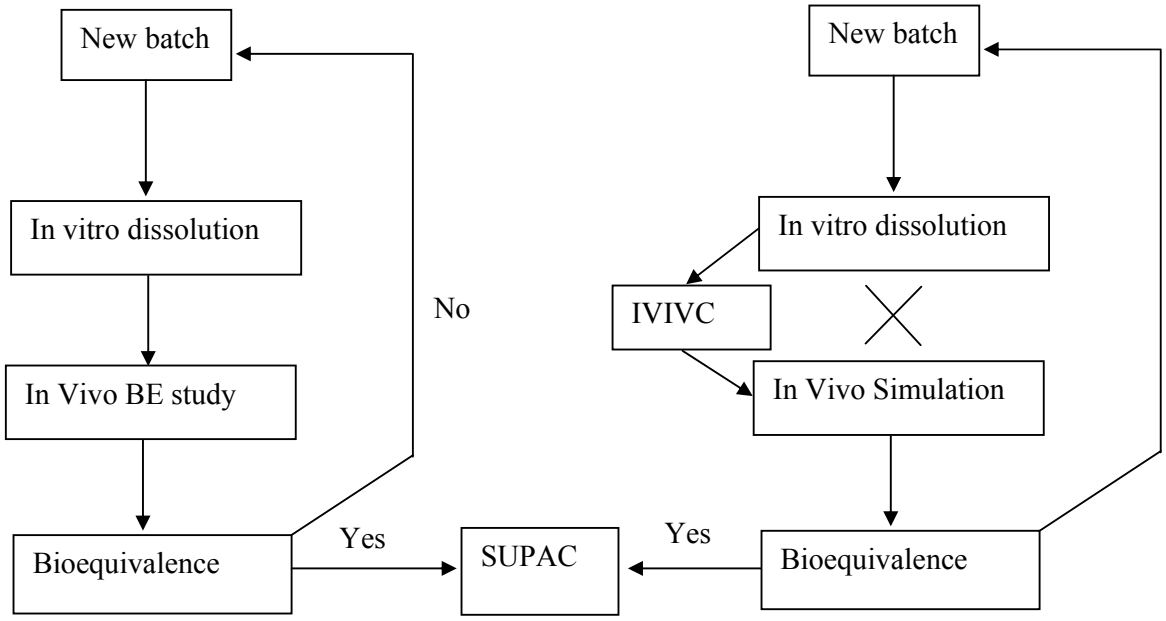


Figure 10: Use of IVIVC as biowaiver

The use of IVIVC as a biowaiver is perfectly described in FDA note for guidance. Those biowaiver involved a validated Level A correlation for extended or delayed release formulation, and could be applied in case of major changes of non release-controlling excipients, minor changes of release-controlling excipients, major changes in method or site of manufacturing. Various cases are described and are function of category and level.

In conclusion IVIVC is a tool that can be used in numerous cases. IVIVC, as a multi factorial tool, needs information from various domains and helps both a communication between collaborators

and an improvement of the knowledge about API and formulation, production process, in vitro dissolution and pharmacokinetics.

In development IVIVC is a tool to understand how the formulation and process behave, to identify critical/key factors of formulation (Release controlling excipients or binder etc.) and of the process (Wet granulation, drying, compression force, etc…). It helps to optimize development and to lower the risk of non-bioequivalence studies even if biowaiver are not used for the dossier. IVIVC could be used to helps production scale up and modification, adjustment of formula, line extension and might also be used post approval as biowaiver (Change in formulation, production site, etc…). IVIVC represents in all cases a gain in the dossier to explain the development rational, to implement quality by design or for regulatory modifications.

For generic industry IVIVC is a tool that can be used, starting from published data, as a first approach of the formulation. This approach could not be used as biowaiver but only as a factor of development speed up and dossier rational.

The IVIVC implementation cost is limited versus the full development but it has limitations like all the tools. The main one being that a correlation established for a type of dosage form cannot be extrapolated to another type of dosage form, the route of administration must be identical for all formulations and the release must be the limiting factor

2.4.2. Dissolution development for IVIVC/R

A basic relationship might be found between API properties and PK data (see also **Paper 1**). This relationship can be in the form of a rank order or can be modeled mathematically (Emami 2006, Cardot 2005). In the second level, deconvolution of PK (e.g. Wagner Nelson or Loo-Riegelman method) (Wagner 1963, Loo and Riegelman 1968) data might be used to establish IVIVC or IVIVR. The relationship can be achieved by correlating the fraction of dose dissolved versus the fraction of dose absorbed, estimated by deconvolution (see also **Paper 2**). In most cases, however, this correlation requires that the absorption process is dissolution controlled. For IR products, this approach mostly fails or, in some cases, requires a scale factor between *in vitro* and *in vivo* data (Löbenberg 2000, Levy 1965). For extended-release products, there is a high probability of establishing IVIVC. When IVIVC cannot be established using deconvolution, convolution-based models should be used. Convolution-based approaches use models such as the Advanced Compartmental Absorption and Transit (ACAT) model or other PK models to predict the oral performance of a dosage form (Wei 2008). *In vitro* data are used in these models to predict the plasma time curves. Such a prediction, if established by using the appropriate parameters, is a Level A correlation.

Determination of IVIVC and IVIVR is a continuous effort throughout development. It requires input of data, including human PK levels and pharmacodynamic properties, food effects, API properties (BCS), and dosage-form information (i.e., excipient properties). Computer tools can be used to develop IVIVC and IVIVR. See also next chapter.

2.4.3. Simulation tools

Many efforts have been made to establish systems simulating in vivo behaviour of formulations. A number of non compendial multicompartment dissolution models have been developed to more closely mimic the in vivo situation. These systems are typically configured to allow transport of buffer contents from gastric compartment to a second intestinal compartment. Some systems, like the TIM model of TNO Pharma (Souliman 2007), attempt to exactly mimic in vivo conditions. For instance it was shown that the TIM-1 could be used to generate a level A IVIVC for paracetamol in both fasted and fed states (Souliman 2006).

But the high complexity of the TIM model makes it impractical for routine dissolution testing. The artificial stomach duodenal model (ASD) which has been used to evaluate the effect of gastric emptying on API dissolution, solubilization and precipitation in a separate duodenal compartment. The in vivo relevance of ASD dissolution profiles is based on the assumption that the

concentration of dissolved drug in the simulated duodenum is proportional to its bioavailability (Castela-Papin 1999, Vatier 1998).

Also uses of software (as mentioned in 2.4) using ACAT model are of great help. Simulation of in vitro dissolution behavior exits as well (DDD Plus from *http://www.simulations-plus.com*) but this software offers for the time being only limited features. Commercial pharmacokinetic modeling software, e.g. IDEA, Stella (*http://www.iseesystems.com*) and Gastroplus® (*http://www.simulations-plus.com),* PK-Sim® *(http://www.systems-biology.com/products/pk-sim.html),*Simcyp® *(at http://www.simcyp.com)* and the TNO integrated software *(http://www.tno.nl)* can be utilized to simulate and predict oral drug absorption in different development phases for drug products. In silico, in vitro and in vivo data from test animal's species can be used to predict absorption properties (Parrot and Lavé, 2002). However, these programs are not transparent; the whole structure of the model and the parameter values are not available. The major aim in the simulation is to combine many variables that are related to gastrointestinal tract physiology and drug and formulation properties, and to learn which are the most critical factors affecting drug absorption.

The pros and cons of absorption modeling have been reviewed in 2009 by Fotaki (Fotaki 2009). Only a self-constructed transparent model enables an interactive learning process. Stella software is user-friendly and can be used to construct compartment models like CAT.

It is important to keep in mind that the simulation strongly depends on the quality of the data (e.g. high low standard deviation) and their interpretation. Again the dissolution with its dynamic aspect is a key element.

2.5. Quality by Design and Dissolution

Traditionally, manufacturing processes are fixed early in development with the intention that material produced from fixed (respectively frozen) processes would be equivalent in quality and that quality is measured by end product testing by fulfilling to specification. Dissolution testing is then used to demonstrate that new batches have similar performance to reference pivotal clinical batches.

The aim of QbD is to make more effective use of the latest pharmaceutical science and engineering principles and knowledge throughout the lifecycle of a product. This has the potential to allow for more flexible regulatory approaches where, for example, post-approval changes can be introduced without prior approval and end-product batch testing can be replaced by real time release. Through this understanding, the process and product can be designed to ensure quality and the role of end product testing is reduced.

During Phase III development, QbD approaches can be used to optimize and finalize the drug-product formulation and manufacturing processes. Compared with conventional development approaches, QbD is primarily devoted to increasing the mechanistic understanding of a formulation and its manufacturing processes, their relationship to product quality attributes, and ultimately, their impact on the safety and efficacy of the product. When a QbD approach is selected, robust product formulation and manufacturing processes should be designed to achieve desired product performance and also relate to desired clinical performance. When the product performance can be appropriately characterized by in vitro dissolution (or a surrogate) test, the dissolution test can be a powerful tool. The dissolution test helps then in the identification of critical quality attributes and critical process parameters. Therefore use of QbD and IVIVC/R will contribute to specifications that have meaning with in vivo performance.

FDA and EMEA are encouraging sponsors to use QbD in the development of their drug products. The principle is outlined in the ICH guidelines Q8 (Pharmaceutical Development), Q9 (Quality Risk Management) and Q10 (Quality System). In November 2009, the FDA published as final the ICH Q8(R2) Guidance on Pharmaceutical Development.

A process is well understood when all critical (direct impact) or key (indirect impact) sources of variability are identified and explained (so called "control space"). Variability is managed by the process design and monitoring. Product quality attributes are accurately and reliably predicted over testing of extreme combinations of all operating parameters for process, equipment, and facilities (so called design space). Prior to this, the relevant knowledge about the drug substance, excipients and process operations were gathered into a so called knowledge space. The QbD interdependencies are outlined figure 11

In practice QbD consists of the following elements (ICH Q8(R2)):

- Define quality target product profile (QTPP).
- Design and develop product and manufacturing processes to meet the target product quality profile (Design space).
- Identify and control critical raw material attributes, process parameters, and sources of variability (CQA).
- Monitor and adapt processes to produce consistent quality over time (Control strategy).

Under the QbD system, pharmaceutical quality is assured by understanding and controlling formulation and manufacturing variables, while end-product testing, including in vitro dissolution, confirms the quality of the product. In the context of dissolution, QbD implies establishing the relationships among raw material properties (such as particle size), formulation variables (excipient levels and grade, aging), process parameters (such as compression force and blending time) see

also figure 5 for detailed parameters, and the target product quality profile. This effort will allow defining the design space. Efficient implementation of QbD requires a biorelevant dissolution test during product development. In a QbD system, product attributes such as particle size or polymorphic form that are previously monitored indirectly via a QC dissolution test are monitored and controlled through the design and control of the manufacturing process (control space). Although QbD does not necessarily directly link to clinical relevance, a thorough understanding of the product properties through QbD enables to choose a dissolution test that may provide the desirable IVIVR for drug release. Thus, under QbD, dissolution testing development should mainly focus on its clinical relevance (see also **paper 1**).

QbD is a systematic approach to product development and process control that begins with predefined objectives, emphasizes product and process understanding and sets up process control based on sound science and quality risk management. QbD is partially based on the application of multivariate statistical methods and statistical Design of Experiments (DoE) strategy for the determination of the process and product design spaces and for the development of both analytical methods and pharmaceutical formulations. Adequate process controls in pharmaceutical manufacture are also required to suffice the current FDA demands, such as PAT (Process Analytical Technology). PAT comprises designing, analyzing and controlling processes by measuring on line critical process parameters and quality attributes. However, the PAT initiative is only one topic within the broader FDA initiative of "Pharmaceutical cGMPs for the 21st century – A risk based approach" (FDA 2004). If product performance is within the design space, dissolution testing may not be needed as a routine test for a finished product specification or could be replaced by other surrogate testing (e.g. NIR).

Additional objective to process understanding is clearly biowaiver. By combining the information mentioned in previous chapters of this thesis, the QbD associated with the BCS (chapter 2.3.2) and IVIVC/R (chapter 2.4) , allows for tomorrow biowaiver new perspective see figures 12 and 13.

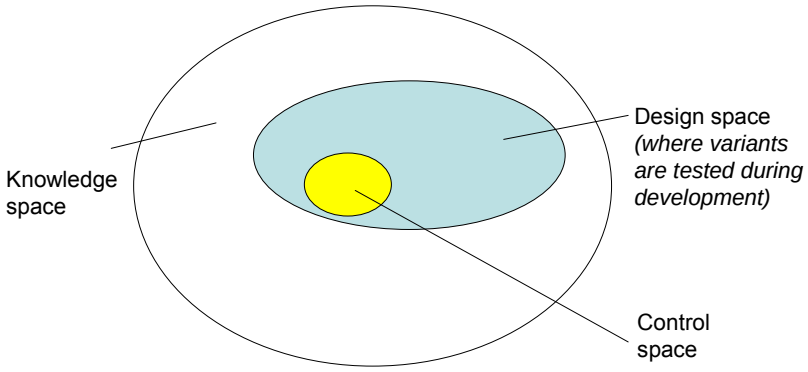


Figure 11: QbD Interdependencies

Despite the many potential benefits, the industry has not been quick to embrace QbD. It is not yet clear how much flexibility regulators will offer, particularly since they have not outlined a clear path for filing beyond the high-level discussion in Q8, Q9 and Q10. In addition, a QbD filing requires a significant level of data sharing (although, in reality, the data must be made available for review if requested, even for a traditional filing). Finally, planning for a QbD filing requires significant investment of time and effort to coordinate information early in development. QbD is an evolving process. QbD requires that we think in a different way to the 3-batches validation paradigm and from early stage development up to post lauch dissolution remains a major method in this approach (see also **paper 1-2-3-4**).

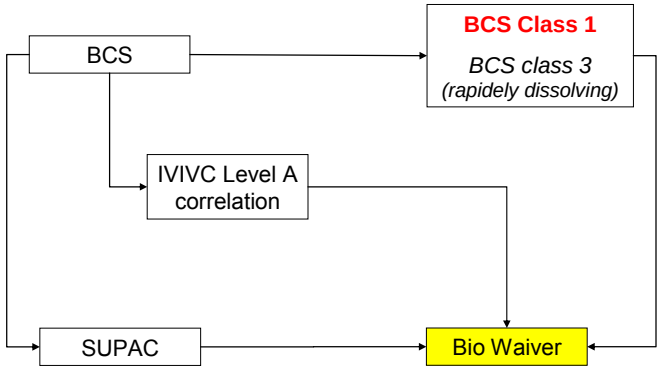


Figure 12: current Biowaiver strategy based on BCS and IVIVC

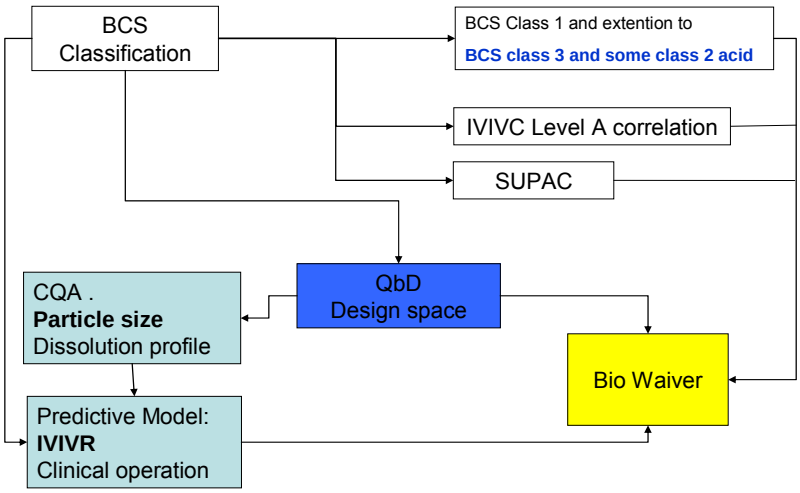


Figure 13: Biowaiver potential for tomorrow (in blue) based on QbD, IVIVC, BCS and SUPAC.

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3. Experimental

Introduction Experimental

Dissolution is a holistic test and reflects on properties of the formulation and API.
The strategy for design of the dissolution testing may vary depending on its utility and the phase of drug development up to a final method for QC. The utility may be to understand the mechanism of drug release for a formulation and its manufacturing processes (API or DP) or to identify any opportunity for an IVIVR/IVIVC or to perform quality control (QC) for process and product consistency. As well dissolution can be used to address biopharmaceutical effect issues for oral formulations like precipitation, which may reduce drug concentration for immediate action, leading to delayed or reduced efficacy (Wei-Guo 2010). In some cases, the method used in the early phase of a product's formulation development might be different from the final test procedure utilized for batch-to-batch control of the product. Therefore, with the accumulation of both in vivo and in vitro experience during a product's development cycle, the early phase method should be critically re-evaluated and potentially simplified. During these phases, however, every attempt must be made to lay a foundation for QbD principles, IVIVC, and IVIVR. The BCS classification of the drug should be established and serve as a guide for the selection of an appropriate dissolution test or surrogate test(s). Ideally, the final QC test should monitor the batch-to-batch consistency of the product and, whenever possible, monitor the key biopharmaceutical parameters or Critical Quality Attribute (CQA) of the formulation. Examples of this approach are presented in this thesis.

The experimental parts of this thesis is presented in forms of a succession of papers, drawing a line thought the different development phases from API selection up to post marketed drug products monitoring and generics. Different aspects of role and importance of dissolution with regard of development of method, discrimination, biorelevance, IVIVC/R in animals and in human as well as QbD and biowaiver for IR and ER formulations are highlighted and commented. The work is structured in fours parts and presented in paper form. Supplements works which were performed later on in the scope of this thesis are integrated as supplement papers.

In a first part the role of the dissolution in the selection of the key parameters for API and formulation are presented. This method was then extrapolated to the use of dissolution to optimize API characteristics leading to early control on product quality via dissolution.
This work is presented as **paper 1** entitled.
"Selection of optimal API properties using in vitro dissolution, animal study and IVIVR to derisk Human study during development."
This paper was not yet submitted and is presented blinded due to some IP confidentialities currently on going.

In a second part the use of dissolution and various media in order to predict in vivo behavior of new ER formulations is presented. This work is presented as **paper 2** entitled.
"**Selection of the Most Suitable Dissolution Method for an Extended Release Formulation based on IVIVC level A obtained on Cynomolgus Monkey"**
This paper has been published in Drug Development and Industrial Pharmacy (DDIP) in November 2010, Vol. 36, No. 11 , Pages 1320-1329
The promising tablets were evaluated on healthy volunteers as PoC. The results are briefly listed and commented in the first supplement (paper 2 Supplement 1) entitled: "**Confirmation of IVIVC in human**". In addition and to support a better process understanding the development of an imaging technology associated with the dissolution was investigated. The experiments are presented in the second supplement (paper 2 supplement 2) entitled: "**Understanding of the release mechanism using imaging technology**".

In a third part the possible use of dissolution in early development phase using non compendia methods have been investigated. The use of small volume vessel and small paddle in place of compendia system is commented using different kind of drug product.
This work is presented as **paper 3** entitled.
"Small Volume Dissolution Testing as Powerful Method during Pharmaceutical Development"
This paper has been published in *Pharmaceutics* in November 2010, Vol. 2, Pages 351-363. Further investigation using the small vessel and the basket method are briefly presented in the first supplement (paper 3 supplement 1) entitled "**Small Volume Dissolution Testing using Basket method".** On other example of applying small vessel non compendial method is highlighted in the supplement (paper 3 supplement 2) entitled "**Tenoxicam-Methylparaben Cocrystal Formation in Aqueous Suspension Formulation** ", where already in early development during the pre formulation, dissolution can support the cocrystall screening program. This work was presented in the AAPS Annual Meeting 2009, poster W4326.

In the fourth part of this thesis, the usage of dissolution to monitor the quality of generic drugs of a Roche product is described with proposal of a simple method that could help to discriminate formulations that might not exhibit similar BE parameters in comparison to the innovator. This work is presented as **paper 4** entitled.

"**Mycophenolate mofetil: use of simple dissolution technique to assess difference between innovator and generic formulations**"

This paper is currently in review process for publication in the journal *: Dissolution Technologies.*

The data are also well abstracted in a poster entitled "***In vitro* dissolution of mycophenolate mofetil: comparison between innovator and generic formulations** "which was presented twice. At BPS Winter meeting 2008, Abstract 0225 and at the ACCP/ESCP International Congress 2009, Presentation 114E.

In conclusion of these investigations, important differences exist between the different generic formulations with regard to *in vitro* performance. In a next step, an exploratory clinical testing was set up to evaluate the pharmacokinetics of different generics that showed the more pronounced difference. The data are presented as a supplement (paper 4 Supplement 1) entitled "**Confirmation of the hypothesis in human** "

All the studies and the importance of having a strong discriminating dissolution methods, IVIVC/R and QbD as well as the general need of having a strategy for brand protection for all drugs already in the early development is discussed shortly in the conclusion .

The experimental parts included in this thesis were carried out at the Pharmaceuticals Division of Galenical and Analytical Development at Hoffmann La Roche Ltd, Basel, Switzerland.

3.1. Experimental part 1

In the first part of these experiments, the role of dissolution in the selection of the key parameters for formulation and process is stressed. IVIVC/R method was implemented to identify API characteristics leading to early control of final product quality using dissolution. The pertinence of the difference observed in vitro was challenged in vivo by screening on monkey and by confirmation with human study. The role of dissolution as a supra indicator respectively global quality tool during development is highlighted and discussed.

This work is presented here as a paper (paper 1) entitled.
"Selection of optimal API properties using in vitro dissolution, animal study and IVIVR to derisk Human study during development."

The NCE investigated was at this time in development phase II and is currently in phase III. Due to some Intellectual Properties (IP) limitations at Hoffmann-La Roche Ltd, the paper was not submitted. It is presented, blinded, in the frame of this thesis in order to discuss the impact of a strong method development design and highlight the role of two dissolution methods applied in early phase on API and DP as tool of QbD.

Original Article

Selection of optimal API properties using in vitro dissolution, animal study and IVIVR to derisk human study during development.

Emmanuel Scheubel [a] *, Valerie Hoffart[b] and Jean Michel Cardot [b]

[a] F. Hoffman-La Roche Ltd, Pharmaceutical Division, Galenical & Analytical Development. CH-4070, Basel, Switzerland.

[b] Univ.Clermont 1, Biopharmaceutical Department,UFR Pharmacie, 28 Place Henri Dunant, B.P. 38,F-630001 Clermont-Ferrand, France

* *Corresponding author*: e-mail: emmanuel.scheubel@roche.com

Key words: API, IVIVC, IVIVR, dissolution, biorelevant specification, particle size, PAT, QbD

Introduction

During the development of new medicines and drug dosage forms the main concerns of the pharmaceutical company is to develop the optimal drug product: therapeutic effect, safety, easy manufacturing, stability and low cost. This development starts from the selection of the most appropriate Active Pharmaceutical Ingredient (API) up to the production of a robust formulation. All the development steps have one goal: to develop an adequate and reproducible dosage form able to guarantee the desired in vivo performance. For a given permeability, the absorption of the substance within the blood flow is governed either by the solubility of the API itself and/or the release of the API from the drug dosage form[1]. For Immediate Release formulation (IR) of oral solid dosage form which by definition exhibited a fast disintegration and thus a fast drug release, the limiting step is the solubilisation of the API. This solubilisation depends mainly on API solubility and dissolution rate. Thus, the solubilisation speed is influenced by intrinsic properties of API solid state such as particle size, crystal shape, and polymorphism. Consequently, any phenomenon able to modify these parameters may have an influence on the API solubilisation and eventually the in vivo behavior of the medicine and should be studied.

Time is an important factor during the drug development and all the possibilities to shorter development time in the respect of the overall safety and quality of the product is a key factor. One tool that is classically used in this race against the clock is *In Vitro in Vivo* Correlations (IVIVC) or *In Vitro in Vivo* Relationship (IVIVR)[2]. IVIVC/R is a tool to predict *in vivo* results based on *in vitro* data that (i) allows to optimize dosage forms with the fewest possible trials in animal or man (ii) fix dissolution acceptance criteria and (iii) can be used as a surrogate for further bioequivalence studies while being a recommendation from regulatory authorities[3456]. In front of development of the IR formulation, IVIVC could be used to select the API form and to evaluate impact of API modification and then to robust the final IR formulation.

This article focuses on a new approach using IVIVC/R to select key factors of API that govern final quality of the IR formulation. A low soluble high permeable drug (class II according to the biopharmaceutical Classification System (BCS[7])) was used as a model. This drug was formulated into classical IR tablet. In order to early identify factors affecting the performance several investigations were performed: (i) in vitro performance to preselect API form for animal studies, (ii) animal studies to decrease the risks linked with human administration (iii) human in vivo study. All the data were further analyzed to identify relevant API parameters and to design space to find the optimal API physiochemical characteristics.

Material and Method

All used chemical were of pharmacopeia or gradient grade quality.

API and formulation

The API is a BCS class II drug exhibiting very low solubility in water. The more relevant parameters influencing its solubilisation were investigated among particle size distribution, specific surface and polymorphism. Four different batches of API , called API A, B, C, D were prepared in order to find the relevant parameters which could insure an optimal exposure for the subjects (Table 1). Batches A, B and C were solely composed of the polymorphic form I. Depending on the desired particle size distribution (PSD), different technologies were used for these tree batches: respectively jet milling, hammer milling and crystallisation. Batch D was composed of 75% of polymorphic form I and 25% of form II. This batch was prepared to evaluate the impact of both polymorph forms with similar PSD. An amount of 25% form II was chosen in order to potentially see an impact on the performance in vivo, assuming some variation of the response, and if it is the case to be in a position to detect it properly. Both polymorph forms having similar solubility and intrinsic dissolution (data not shown).

The PSD was evaluated by laser diffraction using a Sympatec Helos©

The specific surface (BET) was measured using a Micromeritics Tristar©.

Table 1 : Characteristics of the four API batches

API	API A (reference)	API B	API C	API D	
Main technology	Jet Milled	Hammer milled	Crystallization	Jet Milled	
Polymorphic form	Form I	Form I	Form I	Form I 75%	Form II 25%
D 10% [µm]	1.1	3.7	15	1.4	1.1
D 50% [µm]	3.7	27.0	84.3	5.4	3.9
D 90% [µm]	12.2	109.0	177.9	21.2	13.2
BET [m^2/g]	3.2	0.7	0.25	1.8	2.6

BET : specific surface

D particle size to limit the particle size distribution at various percentages

These four API batches were formulated into tablets (called tablets A, B, C, D respectively) by wet granulation using the same composition and process parameters. The four tablets exhibited the same physicochemical properties (e.g.: similar hardness, disintegration, data not presented). The same tablets were used for in vitro studies, animal and human in vivo studies The formulations used in these studies were produced with respect to the current Good Manufacturing Process (cGMP) rules.

Dissolution Studies

Dissolution profiles of the 4 different batches of pure API were performed using an USP4 apparatus (Sotax CE70, Allschwill, CH) equipped with 12 mm cells. The dissolution media was HCl 0.1N containing 0.2% (m/v) sodium dodecyl sulphate (SDS) at a flow rate set at 20 mL per minute in an open modus.. These conditions provide sink condition and acceptable stability during the dissolution testing. Samples were withdrawn at predefined time intervals up to 90 min and were directly filtered using glass fiber filter The amount of drug solubilised at each time point was determined by a validated HPLC-UV method. All experiments were performed in triplicate.

Tablet dissolution profiles were investigated in 1000 mL of the same buffer in an USP2 apparatus (Sotax AT7smart, Allschwill, CH) with rotation speed set at 50 rpm and temperature at 37 ± 0.5°C. Samples were withdrawn at predefined time intervals up to 60 min followed by an infinity testing (15 min at 200 rpm) and were directly filtered. The amount of drug released at each time point was measured by the same validated HPLC-UV method. All experiments were performed with six units.

In vivo bioavailability assessment

Animal studies

A single dose four ways cross over pharmacokinetics (PK) study was performed on 4 Cynomongus monkey. Due to animal's ethical limitations, only limited number of blood samples could be withdrawn and a particular attention was given to the early time points. After oral administration of the tablets (tablet A, B, C or D), the samples were collected at predefined time intervals (0, 0.5, 1, 2, 3, 4, 7, 24, 32 h) and measured by a validated HPLC-UV method. Classical bioavailability parameters were calculated: maximum observed concentration and time to obtain it (Cmax and Tmax) and amount absorbed (AUC).

Human studies

Two selected tablets (tablet A and B) were then further tested in 22 healthy volunteers in fasting conditions in a two way cross over. The blood samples were collected at predefined time intervals from 0 to 24 h and measured by a validated HPLC-UV method.

IVIVC/R

Various approaches of relationship between in vitro and in vivo data based either on values (IVIVC) or rank (IVIVR), were tried on the main bioavailability parameters (Cmax and AUC**).**

Results and Discussion

In vitro results

The dissolution profiles of the different API batches are presented in figure 1.

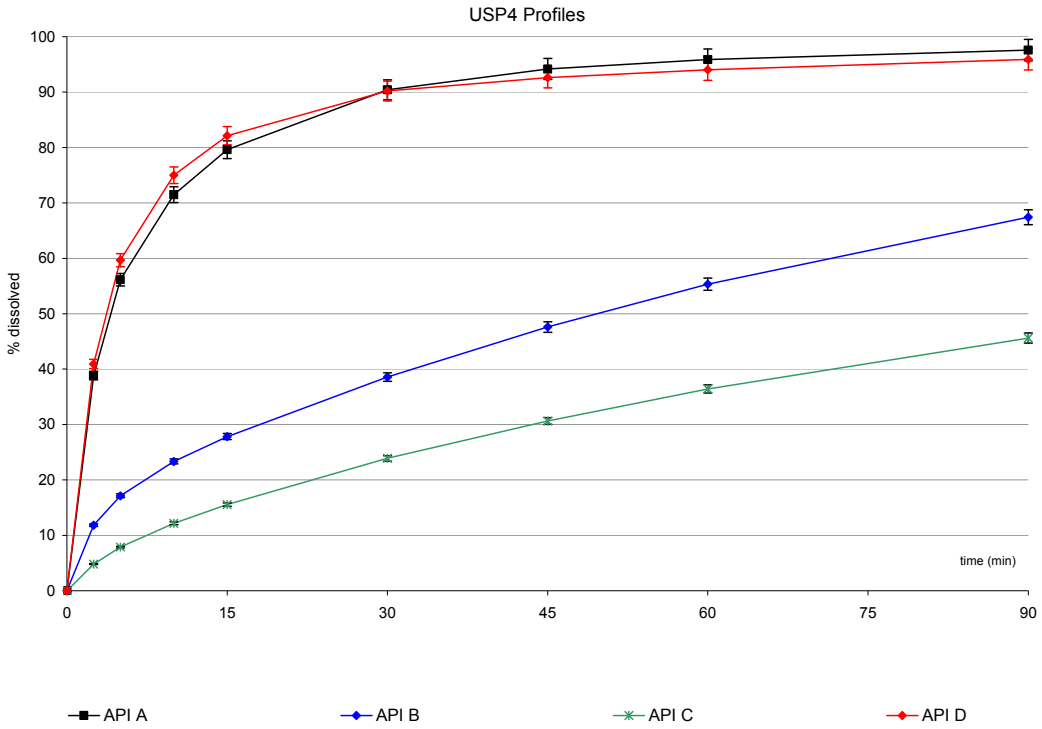


Figure 1: In vitro dissolution of the four API batches using USP4 (n=3, mean values ± SD)

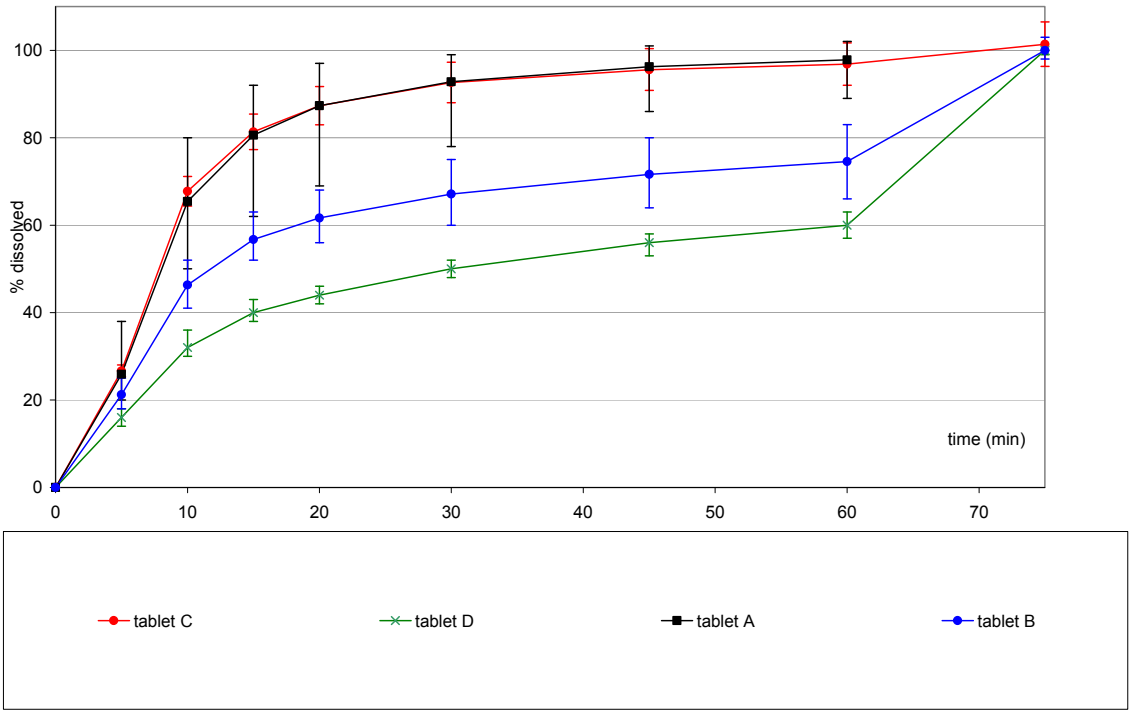


Figure 2: In vitro dissolution of the four tablets using USP2 at 50 rpm (n=6, mean values ± SD)

The API A and D exhibited similar and fast dissolution profiles with more than 80% of the drug being in solution within 15 min, whereas forms C and B clearly showed slower dissolution (68% and 45% dissolved within 90 min respectively).

The release profiles of the different tablets containing various API forms are presented in figure 2. Tablets A and D exhibited similar immediate release dissolution profiles with more than 90% released in less than 30 min, whereas tablets C and B showed slower dissolution profiles. The variability remain relatively high (>10%) after 30 minutes for the tablet A, B and D. However difference in performance can clearly be distinguished between tablets. All samples reached maximum dissolution after infinity testing.

The differences in performance observed for the four tested variants with both methods (USP2 and USP4) are therefore clearly in relation with the solid state properties of the API and less with the formulation. The profiles showed clearly performances in relation with the solid state properties of the API.

In vivo results

The mean monkey *in vivo* pharmacokinetic parameters are presented in Table 2, the plasma time-concentration are presented in figure 3.

Table 2: Mean Pharmacokinetic parameters observed for the four type of tablets in monkeys (n=4, mean value).

Tablet type		**A**	**B**	**C**	**D**
Cmax	**[ng/mL]**	420.0	249.0	153.3	400.0
Cmax/Dose	**[ng/mL]/mg**	108.1	67.8	41.6	108.5
Tmax	**[h]**	5.0	4.5	5.5	5.0
AUCinf	**[ng.h/mL]**	6936.8	5515.3	3061.7	7105.0
AUCinf/Dose	**[ng.h/mL]/mg**	1781.9	1481.6	833.5	1933.0

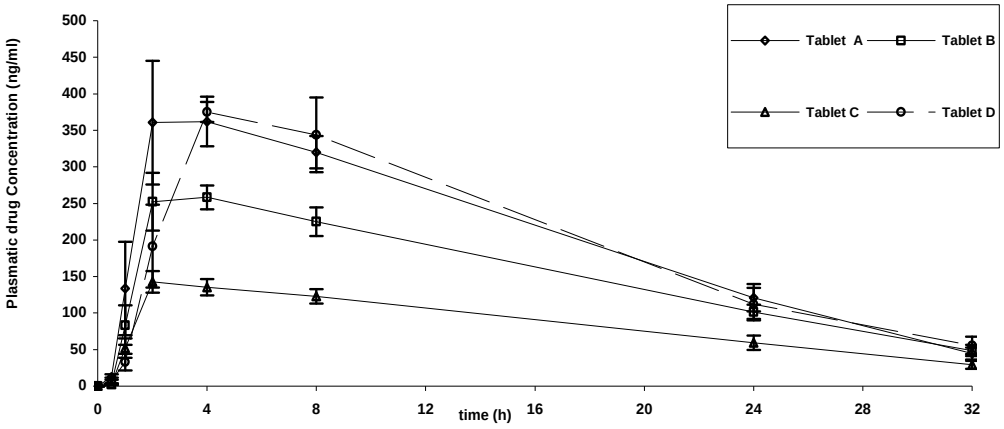


Figure 3: Mean *in vivo* plasma time-concentration data observed in Monkey after oral administration of different tablet type (n=4, mean values ± SD).

After oral administration in monkeys, tablets A and D provided AUC and Cmax not significantly different, respectively 6.9 vs. 7.1 mg.h/mL and 420 vs. 400 ng/mL. In comparison, tablets B and C exhibited smaller AUC and Cmax, respectively 5.5 and 3.1 mg.h/mL and 249 and 153 ng/mL. The four Tmax are comparable.

Based on these monkey's results, the formulations A and B were selected for human studies. Formulation D was discarded as a combination of polymorph which did not impact bioperformance in comparison to A. Tablet C was discarded as being the slowest performer.

The human data are presented in Table 3 and Figure 4.

Table 3: Mean pharmacokinetic parameters observed for tablet A and B in human (n=22, mean values, % Coefficient of Variation (CV) shown in bracket)

Tablet type		A	B
Cmax (CV%)	[ng/mL]	165 (26.6)	102 (21.7)
Cmax/Dose (CV%)	[ng/mL]	5.49 (26.6)	3.39 (21.7)
Tmax (min-max)	[h]	3 (1-7)	3 (1-7)
AUCinf (CV%)	[ng.h/mL]	6940 (26.3)	5430 (30.7)
AUCinf/Dose (CV%)	[ng.h/mL]	231 (26.3)	181 (30.7)

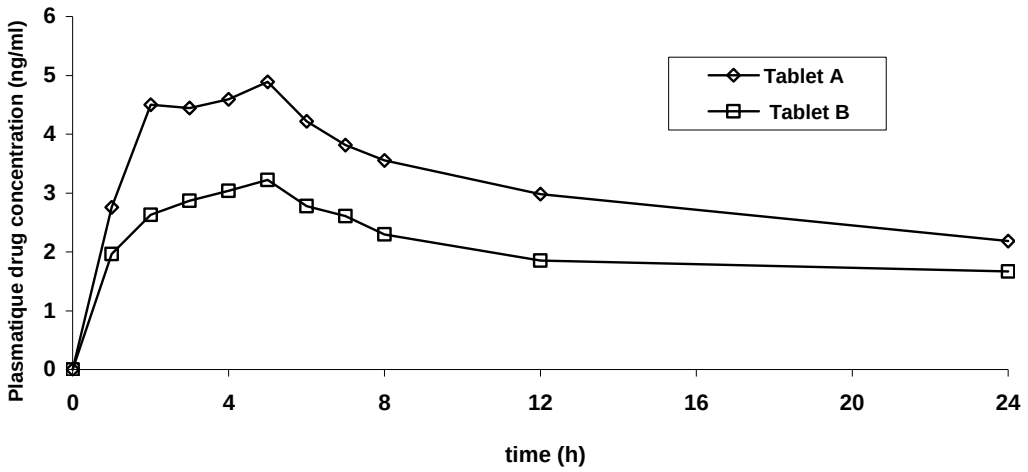


Figure 4: *in vivo* plasma time-concentration data observed in human n=22, mean values)

The pharmacokinetic studies performed in human using tablet A as reference gave the following results: ratio of 78% and 90% CI of [72, 80] for AUC/Dose and ratio of 62% and 90% CI of [57, 67] for Cmax/Dose.

The same performance ranking was found in monkey and human for the formulation A and B containing fine and coarse API. The results confirmed the importance of controlling the drug substance particle size distribution.

In vivo in vitro correlation

Level C was attempted between Cmax and AUC obtained in monkeys and percent of the dose dissolved in vitro at various times. The results are presented in figures 5 and 6 and Table 4.

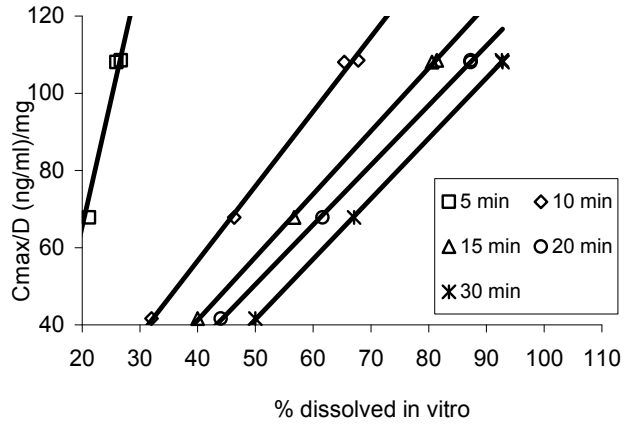


Figure 5: IVIVC observed between percent of the dose dissolved in vitro at 5, 10, 15, 20 and 30 min and Cmax obtained in monkeys.

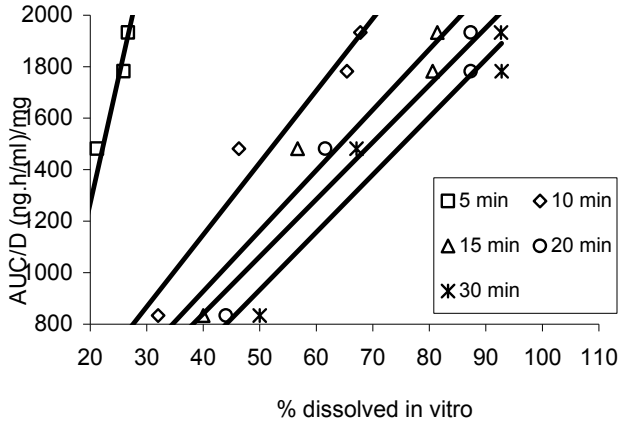


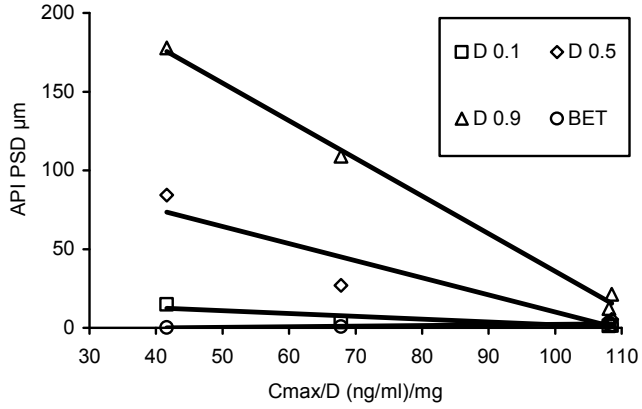
Figure 6: IVIVC observed between percent of the dose dissolved in vitro at 5, 10, 15, 20 and 30 min. and AUC obtained in monkeys.

Table 4: R^2 observed between the main PK parameters obtained in monkeys and the in vitro dissolution.

		R2				
PK parameters	*units*	T= 5	T = 10	T = 15	T = 20	T = 30
Cmax/Dose	[ng/mL]/mg	0.991	0.998	1.000	1.000	1.000
AUCinf/Dose	[ng.hr/mL]/mg	0.990	0.972	0.967	0.965	0.963

The coefficients of correlation (R^2) lie above 0.9 at all sampling times for both PK parameters. A strong relationship was found for the two bioequivalence parameters, Cmax and AUC, and all dissolution time.

Based on this first relationships the main properties of the API (PSD, BET) and the PK parameters (divided by dose in order to be transposable easily in human) was studied and results are presented in figure 8 and table 5



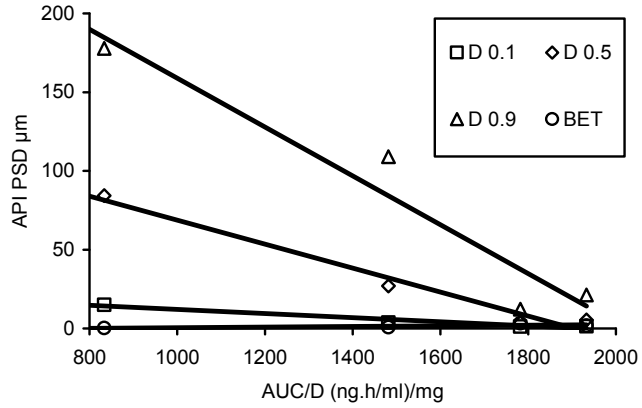


Figure 8: IVIVR observed between percent of the Cmax (8a) and AUC (8b) and API characteristics in monkey.

Table 5: R2 observed between the main PK parameters and the API parameters.

			R^2		
		D (0.1) [µm]	D (0.5) [µm]	D (0.9) [µm]	BET [m2/g]
Cmax/Dose	[ng/mL]/mg	-0.902	-0.943	-0.998	0.884
Tmax	[hr]	0.702	0.621	0.358	-0.140
AUCinf/Dose	[ng.hr/mL]/mg	-0.970	-0.985	-0.963	0.759

The R^2 lie above 0.9 for the PSD associate with Cmax and AUC, whereas lower R^2 are observed for BET. Tmax does not correlate well.

Despite of known difference in transit time and pH, monkey are suitable model for comparison and Proof of Model (PoM) as an understanding of drug and formulation properties[8]. In our case, the drug was tested in animal first to select the API properties to assess reasonable performance before administration in human. In addition the results in monkeys indicated an IVIVC of level C between

the main bioequivalence parameters (AUC and Cmax) and the in vitro dissolution parameters. The results of the IVIVR stressed that D0.5 and D0.9 were overall the main parameters that governed the Cmax and AUC.

As the main parameters for this IR formulation are clearly linked with the API properties, the results obtained in monkeys could be extrapolated to human as the galenical formulation and transit time did not play the major role in this case. That is confirmed by rank and ratio observed between monkey and human which were similar. The ratios of formulation B vs. A were of 62% and 83% for Cmax/D and AUC/D respectively in monkey and of 62% and 78% respectively in human.

In connection with the observed IVIVR and IVIVC (Table 4 and 5) a specification on PSD can be proposed. Based on the results on the human PK study, the parameters used in the calculation of the 90% Confidence Interval (CI) were extracted such as residual variance, degree of freedom for tabulated t and number of subject. The higher and lower dissolution specifications that lead to bioequivalence with API form A were calculated for each PK parameters (Cmax and AUC). This can be done using the 90% CI equation as described in equations 1 and 2, adapted from the concept described by Cutler et al[9] .

Equation 1 Lower Limit: $LL = e^{\left[\left(Ln(\overline{m}_{test1}) - Ln(\overline{m}_{ref})\right) - \frac{t \times s_r}{\sqrt{\frac{2}{n}}}\right]}$

Equation 2 Upper Limit: $UL = e^{\left[\left(Ln(\overline{m}_{test2}) - Ln(\overline{m}_{ref})\right) + \frac{t \times s_r}{\sqrt{\frac{2}{n}}}\right]}$

From those equations, the two "test" means corresponding to the lower (LL) and upper limit (UL) of the 90% CI could be extracted and lead to equations 3 for the lower limit and 4 for the higher limit.

Equation 3: $e^{\left[Ln(LL) + \frac{t \times s_r}{\sqrt{\frac{2}{n}}} + Ln(\overline{m}_{ref})\right]} = \overline{m}_{test1}$

Equation 4: $e^{\left[Ln(UL) - \frac{t \times s_r}{\sqrt{\frac{2}{n}}} + Ln(\overline{m}_{ref})\right]} = \overline{m}_{test2}$

For BE estimation, lower and upper limits could be set to 80% and 125%.

The results of the human PK study led to ratio of 62% and 90%CI of [57, 67] for Cmax and ratio of 78 % and 90%CI of [72, 80] for AUC. Based on this study, the limits to have equivalence between formulations A and B were back calculated. The formulation A was selected as reference, using

equation 3 and 4. The calculations were based on the hypothesis (i) to be bioequivalent (BE) to formulation A and (ii) to include 1 in the 90% CI. Assuming principle (i) the results were, for the lower BE limit [0.80-], an API range of 2µm, 13µm, and 40µm for d0.1, d0.5, and d0.9 respectively. For the higher BE limit [-1.25] the API would result in nano range. Based on the hypothesis (ii), the specification for API were of d0.5< 8µm and d0.9 < 25µm that would result in a BE ratio of 96 and 90% CI of [92-101] for AUC/D and 93% and 90% CI of [92-100] for Cmax/D. Those findings are stricter than the API form B PSD and the calculated limits can then be reached only using the jet milling and not the hammer milling technology.
Based on this range, the dissolution specification, set at a single time point, was back calculated. The dissolution limits, which can insure bioequivalence, were calculated to be greater than 80% at 30 minutes.

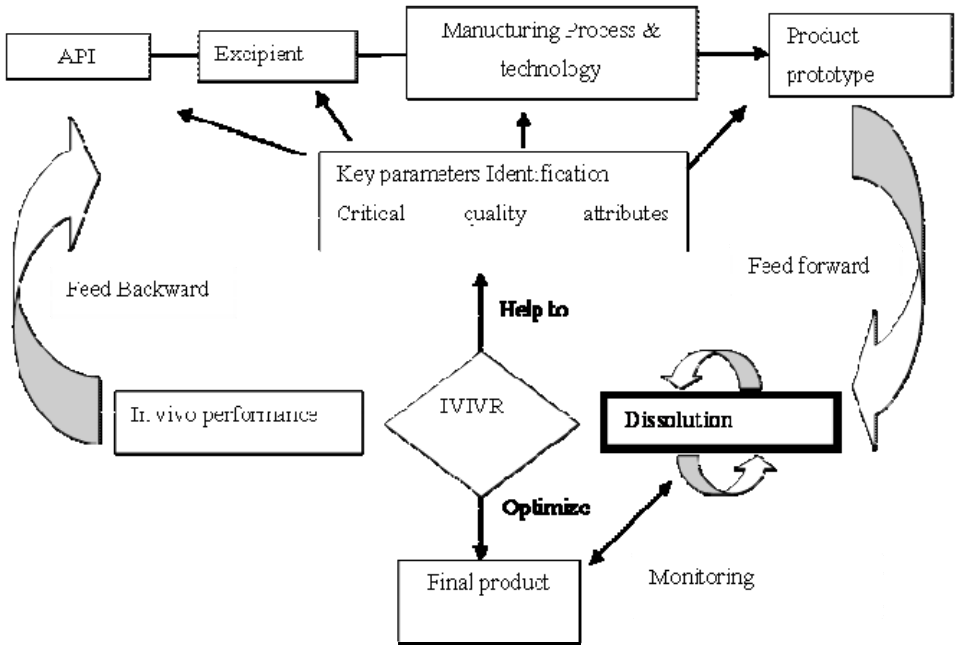
The technique of dissolution limit settings on the final formulation could also be applied to the raw material, API, allowing accepting or rejecting the batches before any further formulation and manufacturing steps or by derisking the scale up of API batches. The impact of further manufacturing steps on the dissolution would have to be checked before setting dissolution limits on API. As a target, based on figure 1, a specification at 80% dissolved after 90 minutes for the API should ensure, if the tablets performance are as well within specification, to fullfill the BE criteria. This dissolution held on API could be considered as an in process control (IPC) at entry of the raw material within the production line and can be assimilated to a control close to a process analytical technology (PAT) method as this simple and unique control (in addition to identification and purity) could discriminate the overall qualities of the active substance to comply with the characteristics of the final product.
The dissolution method applied on the API allows monitoring the performance based on the PSD. API with similar PSD should result in similar profiles. If differences are observed, for instance after scale up, process optimisation or change in final step (e.g. drying, milling), other properties should be checked (e.g. BET, wetability, bulk density) before manufacturing of the tablets and further in vivo testing. As well if a low dissolution of a new tablet batch is observed, the successfully previous control of the dissolution rate of the API will rather indicate an issue during manufacturing of the tablets.

IVIVR can also be used, in contrast to IVIVC, to identify the key parameters of the formulation, process or API in which the release is not the limiting factor. Therefore before further in vivo test, the dissolution performance can serve as monitoring tools to identify the most suitable API batches. Those relations are a first step to implement Quality by Design (QbD) and design space by

monitoring of the Critical Quality Attributes (CQA) that are likely to impact in vivo performance and are in accordance with the concepts developed in ICH Q8[10].

Dissolution on drug product was used in this example as a surrogate marker or a supra indicator of all processes which are involved in the quality of the API, formulation and manufacturing process. This approach allows the key factors to be followed either through their direct monitoring or their impact on dissolution. Figure 9 sums up the importance of IVIVR and dissolution on the optimisation and follow up to the key parameters throughout the development.

Figure 9: Dissolution and IVIVR as a Total quality Tool



By a feed forward and feed back control, a design space can be established where the identification, characterization of critical-quality attributes and identification of root causes of variability are the main activities and must lead the adaptation of the drug product manufacture. Even if dissolution is not an on-line tool for measuring quality on the production lines, it can be considered as a supra indicator which reflects the global performance of all the modifications of

either the API, the formulation or the manufacturing process and can then guarantee the overall pharmaceutical quality of the final product by control of the critical quality attributes (CQAs)[11].

In the current study dissolution, animal and then human data were a help to identify the PSD as the CQA of the pure API. Equivalence needs to be established between batches to insure that bioequivalence would only be linked with the manufacturing process of the final formulation and not any more only to the initial quality of the API.

As the animals' findings were confirmed afterward in human. The relevance of the confirmed difference in human performance will allow (1) to choose the best solid state properties to meet the maximum exposure (2) to select the most suitable milling technology and take into consideration the optimal cost of good (CoG) if milling for instance is not necessary to reach maximum exposure (3) to cross validate the monkey as suitable model for this compound (4) to set up the suitable in vitro analytical method(s) to accurately measure the material in quality control (5) to set up biorelevant specification.

Conclusion

Dissolution and IVIVR are explanatory tools which by identification of key parameter(s) that are likely to influence the performance allow improving the know-how about API intermediate, formulation and process but also development and then derisking in vivo human BA /BE studies by a fine and accurate selection of the variants to be tested in vivo. It has to be kept in mind that the attempt of IVIVR/C, allows sound rationale for API, drug product, dissolution description and setting and supports further scale up and formulation optimization including a biowaiver approach. IVIVC and most likely IVIVR are straightforward tools that can be used when we are in presence API related issues, immediate release formulations and process related problems. It is a help to establish QbD and to better understand the formulation.

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Conclusion experimental 1.

This example demonstrated how early dissolution could drive the parameter setting on API characteristics and on process optimization leading, as mentioned in PAT and QbD, in a selection of more appropriate tests during the production. The application of an USP4 method on API, in addition to the standard QC USP2 for the final tablets, allows to clearly follow the process step by step and identifies at which level change potentially occurs. The USP4 method is suitable also for intermediates as granulate of final blend. In our case the difference observed on the API was the key drivers and the particle size is a clear CQA for this compound as the drug product form did not impact the difference in performance. This difference being biorelevant the approach set by testing first the hypothesis on monkey and after by confirming on human allows defining a strong development strategy. The monkey being then a valid animal model for the human performance. Moreover it places the dissolution applied on API as a strong monitoring tools what will serves for derisking of further development step. This approach is in line with QbD and allow by setting of biorelevant specification, to defined control space and ultimately the overall control strategy.

The overall goal of either establishing an IVIVC/R or implementing QbD is to have a better control of the product performance within the life cycle of a product. Biowaivers are currently only granted for BCS 1 drugs in US (and additionally for BCS 3 under specific circumstances in EU) to establish bioequivalence and if a Level A IVIVC was established. For all other cases SUPAC applies for formulation changes. The current guidelines allow minor changes without the requirement to prove bioequivalence clinically. However, using dissolution and QbD there is no scientifically justification not to grant biowaivers for formulation changes if the parameters in the design space are properly defined and monitored. Reliance on end product quality testing alone doesn't assure enough quality. This can be in particular helpful in case of scale up or modification of strength, change which anyhow occur during the development phase.

3.2. Experimental part 2

A further example of the implementation of dissolution as surrogate for performance of tablets is presented on the paper 2. The use of dissolution and various media in order to predict in vivo behavior of MR formulations is discussed.

After in vitro screening, formulations were tested in monkey in order to assess the absence of risks in human. IVIVC was assessed and the parameters which are likely to impact the performance were identified and discussed.

The data are presented as a paper. The paper entitled "**Selection of the Most Suitable Dissolution Method for an Extended Release Formulation based on IVIVC level A obtained on Cynomolgus Monkey"** was accepted by Drug Development and Industrial Pharmacy (DDIP). (2010, Vol. 36, No. 11 , Pages 1320-1329)

After proof of manufacturability (PoM) confirmed in monkey, the promising tablets were evaluated on healthy volunteers as PoC. The results are briefly listed and commented in the first supplement (Supplement 1) entitled: "**Confirmation of IVIVC in human**". The IVIVC can be confirmed and allows to stronger the found dissolution method as biorelevant tool to support development.

The understanding and control of the release mechanism is further supported by the development of an imaging technology associated with the dissolution. The experiments are presented in the second supplement (Supplement 2) entitled: "**Understanding of the release mechanism using imaging technology**". The combination of both techniques (dissolution & imaging) is discussed briefly in the context of QbD in order to highlight the strength of this association. In this case the main objective was to qualitatively confirm the main parameter impacting the release behavior found to be important for bioperformance.

Selection of the Most Suitable Dissolution Method for an Extended Release Formulation based on IVIVC level A obtained on Cynomolgus Monkey

Emmanuel Scheubel [a, b] *, Laurent Adamy [a], Valerie Hoffart [b] and Jean Michel Cardot [b].

[a] F. Hoffman-La Roche Ltd, Pharmaceutical Division, Galenical & Analytical Development. CH-4070, Basel, Switzerland.

[b] University of Clermont 1, Biopharmaceutical Department, UFR Pharmacie, 28 Place Henri Dunant, B.P. 38,F-630001 Clermont-Ferrand, France

Corresponding author: e-mail: emmanuel.scheubel@roche.com; Tel: +41616886357

Keywords: Extended Release, dissolution, IVIVC, BCS class 2.

Abstract

Objective:

The purpose of this study aims primarily to identify the most suitable in vitro dissolution method(s) for their ability to predict the in vivo performance of extended release (ER) prototype tablets formulations designed for a new chemical entity (NCE), Biopharmaceutic Classification System (BCS) class II drug, weak base, based on data collected in cynomolgus monkey.

Materials and methods:

Different types of buffer at different pH were selected as dissolution medium resulting in a broad variety of release patterns (slow to fast). The in vivo and vitro data were put in relation.

Results:

As a consequence of the discrimination between both tested formulations, the in vitro in vivo correlation (IVIVC) qualities and shapes changed significantly. The obtained level A showed that the simple HCl medium was superior to biorelevant media and media containing surfactant when investigating IVIVC's in cynomolgus monkey. In addition the results of the dissolution in HCL suggested rather a diffusion mechanism of the ER matrix formulation as the main factor of the release.

Conclusion:

Adjusting dissolution testing conditions to match the behavior of the formulations in vitro with that in vivo by taking into account the properties of the drug and the formulation, is a straightforward and useful approach in identifying a predictive method in development of in vitro-in vivo correlation. These investigations will definitely help by derisking of new formulations as well as by rating changes in existing formulations with regard to their impact on bioavailability (BA) before entry into human (EIH)

.

Introduction

A common challenge of all pharmaceutical companies is the development of new drugs, as fast as possible, to cover unmet medical needs and to ensure at the same time safety and efficacy. Many strategies exist and amongst them, in vitro dissolution, animal experiment and in vitro in vivo correlation (IVIVC) can be used early in the development phase in order to minimize the risk before administration into man, to address impact of changes in existing formulations with regard to bioavailability (BA) and to improve the development strategy leading to a faster time to market.

In the present study this approach was used to select an extended release (ER) formulation of a NCE, weak base classified as a poorly soluble and highly permeable drug (BCS Class 2 (Yu, Amidon et al. 2002)). This drug, after oral administration of an immediate release (IR) capsule in human, exhibited side effects, hypothetically due to high plasma concentration. To address this high concentration (Cmax), prototype formulations of extended release hydrophilic matrix tablets were developed and optimized using dissolution techniques in order to sustain Tmax and lower Cmax. During this development, dissolution data were generated in order to assess the formulations performance throughout the optimization process. However, at this stage, the discriminative power and the effectiveness of the dissolution method as a predictive tool to derisk human in vivo study is unknown. Therefore, the development of the initial dissolution method for this poorly soluble compound included the assessment of relevant physical and chemical properties of the drug as well as the key factors of the drug product and formulation. The two optimal formulations, with regard to dissolution performance observed in the simplest media achieving sink condition, were tested in animals before any new administration to humans in order to demonstrate its technical feasibility and efficacy. A systematic screening of various classical dissolution media keeping apparatus and condition similar was realized to better understand the behavior of these two formulations and a relationship between in vitro and in vivo animal data was assessed.

The combination of these tools (dissolution, animal data and correlation as well as some weakness of in silico data) to develop, and select the most suitable in vitro method is discussed in the present paper as a smart development tool to speed up the realization of new formulations and to ensure the best performance during the future human trials.

Materials and methods

Materials

Egg lecithin (E PC S, purity >96%) was obtained from Lipoid (Ludwigshafen, Germany) and sodium taurocholate (NaTC, 97% pure) was used as received from Prodotti Chimici e Alimentari SpA, Basaluzzo, Italy

Phosphate buffer, sodium chloride (ACS), 37% hydrochloric acid (fumed), 85% ortho-phosphoric acid, ethanol (99.9%) as well as HPLC grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Tris buffer was obtained from Applichem (Darmstadt, Germany) and water was obtained from a Milli-Q (Millipore, Milford, MA, USA) water purification system.

The various surfactant; sodium laury sulfate (SDS), hexadecyl-trimethyl-ammonium bromide (CTAB) and polysorbate 80 (Tween 80) were purchased from Sigma-Aldrich Chemie GmbH, (Steinheim, Germany).

Model compound and solid formulations

The poorly soluble NCE Roche compound (RO-X) is a small molecule, a weak base used in the form of hemi-sulphate salt. Only one polymorph form is known and is stable in water. Its main physicochemical properties are ClogP of 3.7, pKa of 4.6. The experimental dose was set at 1 mg.

Experimental immediate release (IR) capsule formulation and extended release (ER) tablet formulations were supplied by Roche Pharmaceutical Research department. The ER formulations were produced by wet granulation using the same batch of API. Different amount of HPMC were adjusted in order to decrease the Cmax having a target of 85% released in vitro within 4 hours or 8 hours respectively. The IR reference formulation exhibits a complete release within 0.5 hour. All formulations were designed and homotetically adjusted for administration in monkey.

Methods

Dissolution media preparation

Various media were tested from pH 1.1 to 6.5. Compendial media were prepared according to the United States Pharmacopeia. Phosphate buffer pH 4.0 and pH 6.0 were prepared from 0.05M sodium dihydrogen phosphate. pH was adjusted using 0.2M sodium hydroxide (NaOH) or phosphoric acid. Surfactant amount was added accordingly. Due to the low solubility, addition of three types of surfactant (anionic, cationic and non ionic) were tested at pH 6.0 in order to have (i) a pH close to neutrality (ii) a pH more in accordance with gastrointestinal (GI) tract pH (Dressman, Berardi et al. 1990; Kalantzi, Goumas et al. 2006), (iii) a similar effectiveness of all the surfactants and (iv) a reasonable stability of the solutions after filtration. Biorelevant media FaSSIF and FeSSIF were prepared according to current procedures (Galia, Nicolaides et al. 1998). Overall, media were stable and present as a single phase. Two steps were included in the dissolution method. Step 1 was handled in the selection of the best formulations to be tested in vivo on monkey and correspond to acidic media alone, step 2 after

having the in vivo results corresponded to the screening of various dissolution methods in order to seek for the most pertinent IVIVC.

Solubility Studies

The solubility of the RO-X was assessed in each dissolution medium. Duplicate samples were incubated with an excess of compound in a 10 ml volumetric flask at 37°C with constant rotation. Samples were collected after 4 hours and 24 hours , filtrated and subsequently diluted with the HPLC mobile phase. The dissolved quantity was measured with a validated HPLC-UV detection method. The solubility studies were carried out with the same batch of API which was used for manufacturing of the tablets.

Dissolution Studies

The dissolution profiles of the ER formulations were examined in the different media, using a Sotax AT7 *smart* apparatus equipped with automated sampling (Sotax, Allschwill, CH). In all cases, paddle speed and temperature were set at 50 rpm and 37°C, respectively with n=3 units. Due to the low dose of the tablets (1 mg) and sensitivity of the analytical method, the dissolution volume was held constant at 500 ml (minimal volume insuring a homogeneity and reproducibility of the media). Ten 1 ml samples were withdrawn at predefined time intervals up to 8 hours from each vessel and not replaced. 20 ml of the test solution were pumped through the circuit before each sampling time to prerinse the sampling lines and filters. Sampling and filtration were automated and dissolution samples were directly filtered and subsequently measured by a validated HPLC-UV detection method.

In vivo bioavailability assessment

A pilot study based on a single dose, 3 arms simple study comparing the 2 ER tablets formulations (target 4 hours and 8 hours release) with the immediate release formulation (reference capsule) was performed on 3 cynomolgus monkeys in fasting conditions. Due animal's ethical limitations, only limited number of samples could be withdrawn and a particular attention was given to the early time points. The samples were collected at predefined time intervals (0, 0.5, 1, 2, 3, 4, 7, 24, 32 hours) and measured by a validated HPLC-MS method.

In Vivo-In Vitro Correlation

For the in vivo data, in addition to the classical bioavailability parameters Cmax, Tmax, AUC, the percentage of fraction of drug absorbed (% FD) was determined by deconvolution using the Wagner Nelson method (Wagner and Nelson 1964; Dressman and Lennernäs 2000)[9]. In vitro the percent of drug dissolved (% D) were obtained from the dissolution. Various approaches of relationship between in vitro and in vivo data were examined based either on values (IVIVC (EMEA 1999; FDA 2002)) or rank (IVIVR (Cardot 2006; Emami 2006)[9]). The in vivo and in vitro data were put in relation using a point-to-point relationship between the in vitro dissolution and the in vivo input of the drug (IVIVC Level A). Linear regressions were primary sought. In case of faster dissolution than input, a scaling factor from linear Levy plots (Levy 1964) or from non linear scaling (Sirisuth, Augsburger et al. 2002) was discussed.

Model predictability was estimated internally by comparison of prediction errors for pharmacokinetic parameters, Cmax, Tmax and AUC, derived from mean observed and predicted in vivo data obtained by using inverse of WN method (Gohel 2005). For a reasonable IVIVC, regulatory guidelines state prediction errors for Cmax and AUC should not exceed 10% (Eddington, Marroum et al. 1998; Sunkara 2003). All calculations were done using Microsoft Excel.

Results

Solubility Studies

The equilibrium solubility at 37°C, over the physiological pH range using classical dissolution media after 4 hours and 24 hours is presented in table 1.

The molecule exhibits a typical pH depending solubility profile of a weak base with a low solubility at high pH. The solubility in the biorelevant media (FaSSIF and FeSSIF) was estimated to be of 12.5 mg/500 ml and 60 mg/500 ml respectively, denoting an improvement of roughly 30 times at pH 6.5 in FaSSIF and of around 140 times for FeSSIF compared to classical pH 6.0 phosphate buffer. Addition of chemical surfactants of either nature between 0.5 % and 1% in dissolution media at pH 6.0 led to solubility estimated to be greater than 7.5 mg/500 ml (increase of solubility more than to 18 times). Acid pH, biorelevant media and media with adjunction of surfactant exhibit sink conditions for the 1 mg dose that being not the case of pure phosphate buffer pH 6.0. Overall, no shifts in pH or precipitation were observed during equilibrium solubility determinations.

Paddle dissolution studies

Six different ER tablets with different amount of HPMC (formulation N° 1 to 6) were first measured by dissolution for pre screening using HCl 0.1N (step1). The IR formulation is presented on those curves as a reference (Fig 1). Formulations N°2 and N°4 exhibited profiles closer to the targets and were selected. Both tablets were further tested using the seven dissolution media (see table 2). The results of the dissolution tests performed with various media (step 2) on formulation N° 2 and N° 4 are presented in Fig 2 and Fig 3.

Dissolution of both chosen formulations (N° 2 and N° 4) exhibited the expected rank order, irrespective of which medium was employed. Standard deviations were observed in a range of 3% to 5%. No coning or mounting was observed during the dissolution tests.

Table 1 : Solubility of Ro-X in the various dissolution media over the physiological pH.

Medium	pH	mg/ml
HCl 0.1N	1.1	13
Phosphate 0.05M	4.0	1.4
Phosphate 0.05M	6.0	0.0008
FeSSIF	5.0	0.12
FaSSIF	6.5	0.025
Phosphate 0.05M + 0.5% SDS	6.0	> 7.5
Phosphate 0.05M + 1% CTAB	6.0	> 7.5
Phosphate 0.05M + 1% tween	6.0	> 7.5

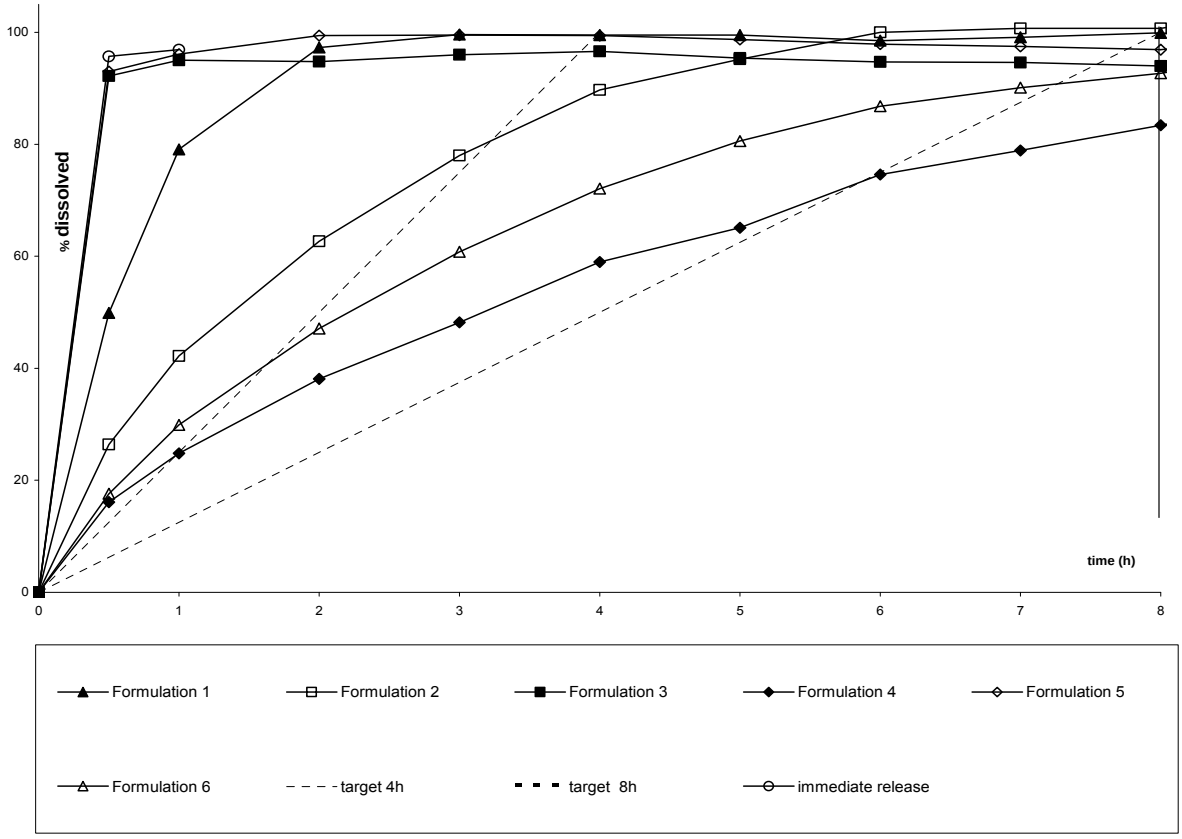


Figure 1: Dissolution Profiles of different ER tablets vs IR formulation in HCl 0.1N

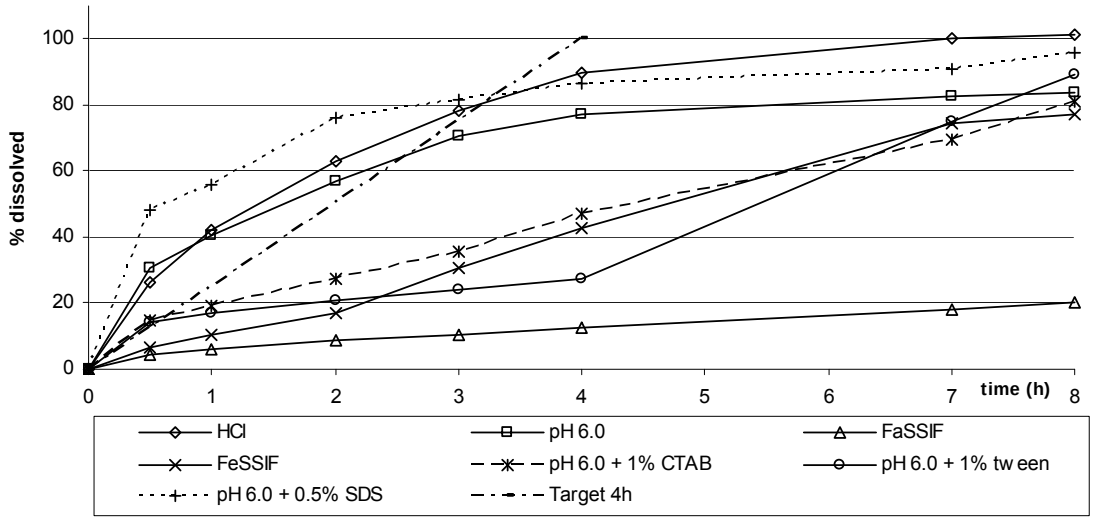


Figure 2: Dissolution Profiles of formulation 2 (ER 4H) within all tested media

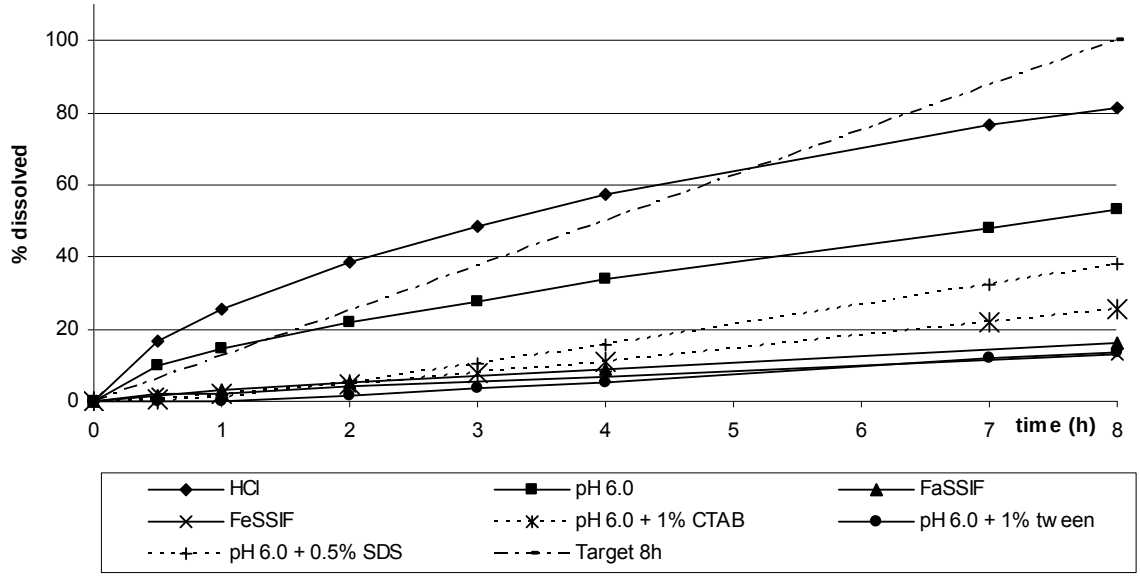


Figure 3: Dissolution Profiles of formulation 4 (ER 8H) within all tested media

In vivo-data

The in vivo Cynomolgus monkey results are presented in figure 4 and in table 2 for PK parameters.

The in vivo release data demonstrated that compared to the reference (immediate release capsule), a lower Cmax and a prolonged Tmax can be observed with the extended release (ER) tablets indicating a slower absorption and an impact of the composition of the tablets on the global performance. The two slow release formulations exhibited a monophasic decline indicating an apparent one compartment model. In this case the Wagner (Nelson Wagner, 1964) method can be used for the deconvolution and the results are presented in figure 5 up to 7h (100% of absorption being reached later). The figure 6 presented the percent remaining to be absorbed denoting an apparent first order kinetic for both ER formulations.

IVIVC

The basic comparison of the dissolution data and the in vivo data indicated a correct ranking of both formulation independently of the media used.

The various In vitro–in vivo correlations level A attempted with the different media tested are presented in figure 7a to 7g and in table 3a, b and c.

No IVIVC of Level C or IVIVR were further investigated as a level A IVIVC can be established.

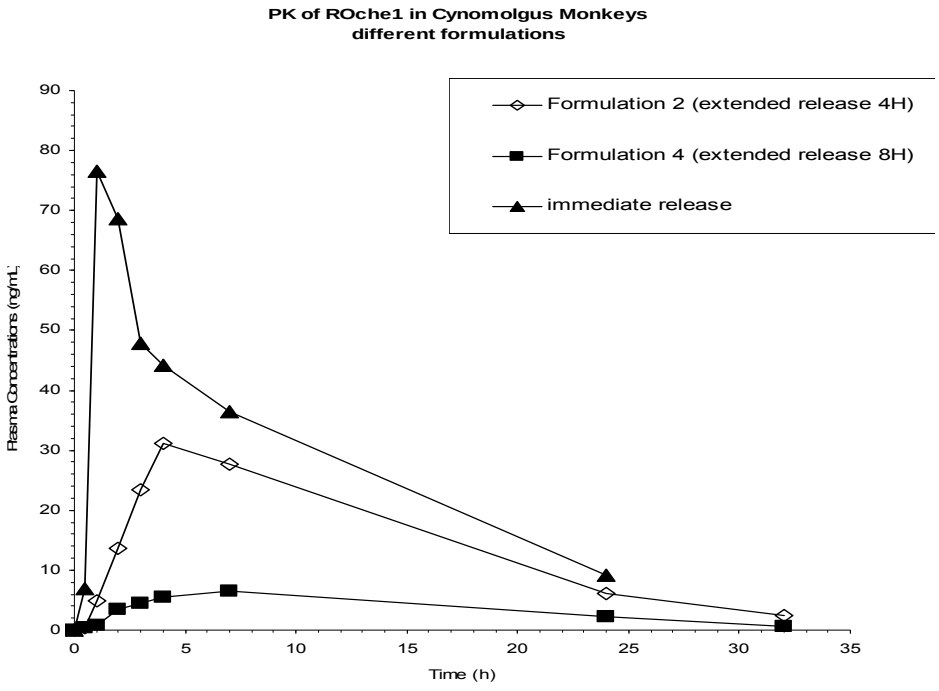


Figure 4: PK mean profiles of RO-X in Cynomolgus Monkeys for the 3 tested formulations (ER formulations 2 and 4 versus reference IR formulation).

Table 2 : Mean (±sd) PK parameters of the RO-X on Cynomolgus monkeys for the 3 tested formulations (ER formulations 2 and 4 vs reference IR formulation).

Parameter	Unit	Formulation 2 ER 4H	Formulation 4 ER 8H	IR Formulation
Cmax	[ng/ml]	36.4 *(±17.6)*	6.47 *(±5.2)*	79.1 *(±9.0)*
Tmax*	[h]	4	7	1
AUC(0-inf)	[(ng·h)/ml]	464 (±145)	114 *(±80)*	847 (±218)

* median

Table 3a: Overview of the obtained r squared after IVIVC level A attempts

R^2	HCl 0.1N	pH 6.0	FaSSIF	FeSSIF	pH 6.0 + CTAB 1%	pH 6.0 + Tween 80 1%	pH 6.0 + SDS 0.5%
Form N° 2	0.97	0.96	0.96	0.93	0.95	0.73 (0.99)*	0.98
Form N° 4	1.00	0.99	0.96	0.99	0.94	0.89	0.90
Forms N° 2+4	0.90	0.67	0.87	0.63	0.64	0.48	0.24

**Value without burst effect after 4 hours R^2 = 0.99*

Table 3b: Overview of the obtained slope after IVIVC level A attempts

slope	HCl 0.1N	pH 6.0	FaSSIF	FeSSIF	pH 6.0 + CTAB 1%	pH 6.0 + Tween 80 1%	pH 6.0 + SDS 0.5%
Form N° 2	1.051	1.411	5.967	1.136	1.449	1.125	1.651
Form N° 4	0.960	1.506	6.215	7.431	2.770	4.819	5.081
Forms N° 2+4	0.885	0.802	5.135	0.958	0.997	0.879	0.720

Table 3c: Overview of the obtained intercept after IVIVC level A attempts

intercept	HCl 0.1N	pH 6.0	FaSSIF	FeSSIF	pH 6.0 + CTAB 1%	pH 6.0 + Tween 80 1%	pH 6.0 + SDS 0.5%
Form N° 2	-33.32	-47.74	-23.55	2.15	-15.06	2.61	-84.32
Form N° 4	-13.47	-10.52	-7.81	-7.96	5.72	9.45	3.40
Forms N° 2+4	-16.28	-1.63	9.02	+13.40	10.05	16.54	14.34

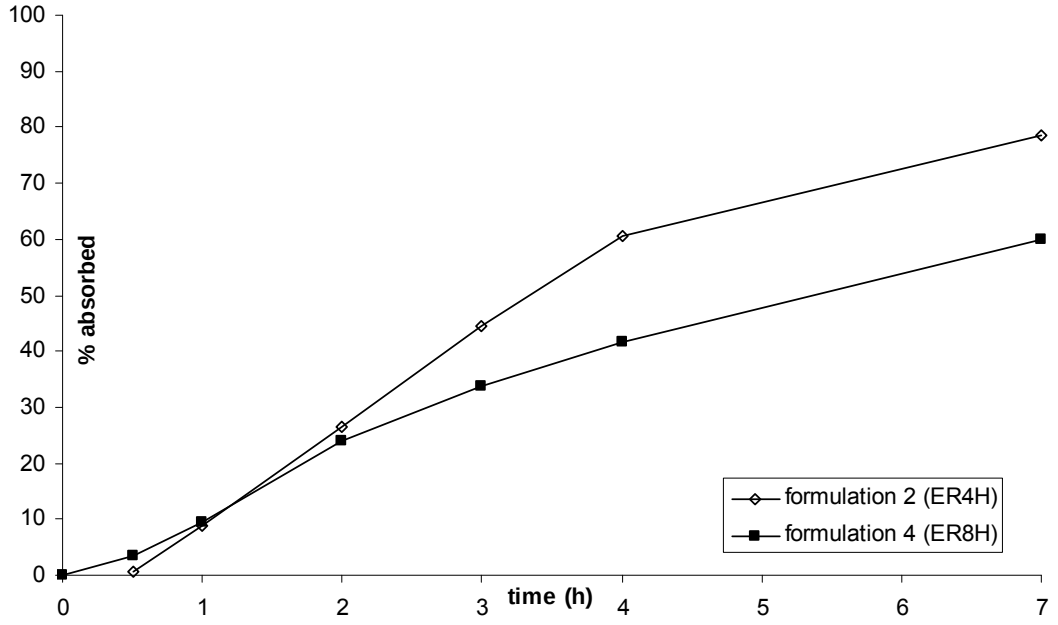


Figure 5: % of fraction absorbed in function of time according to Wagner Nelson

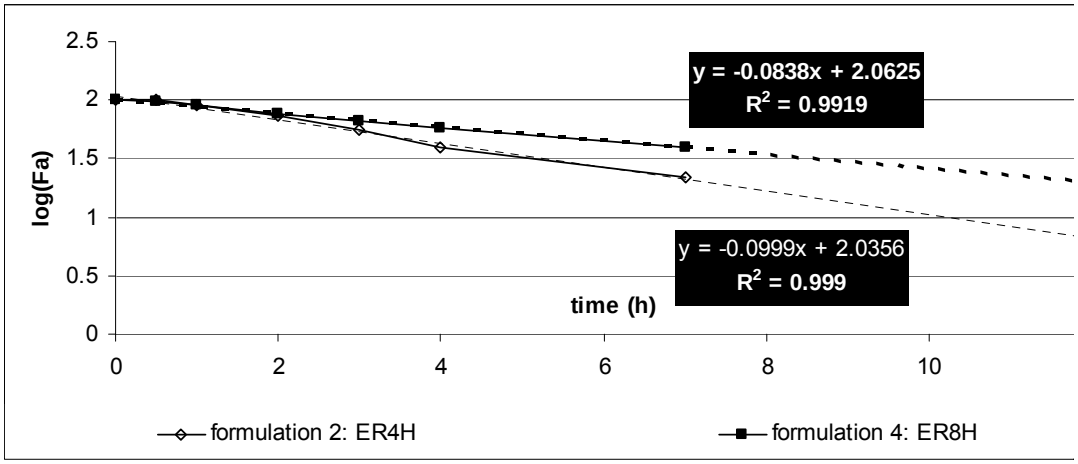
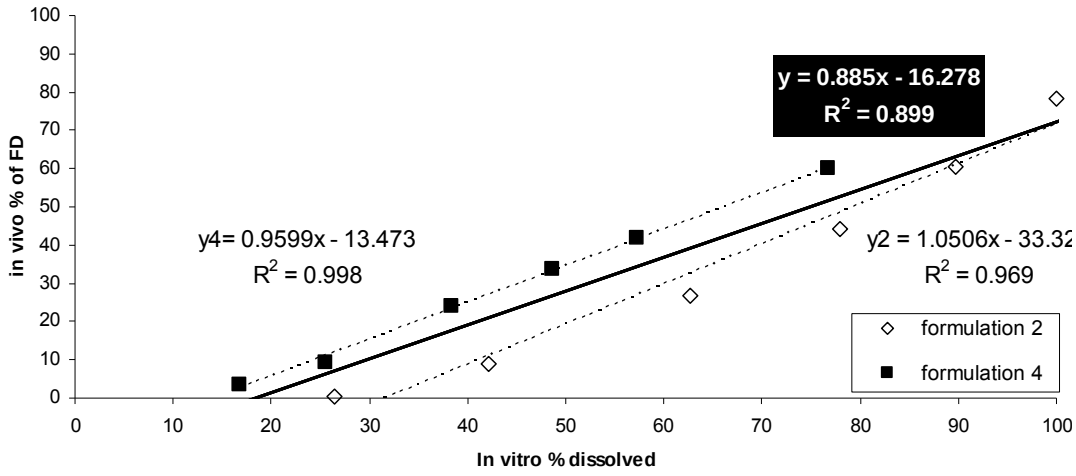
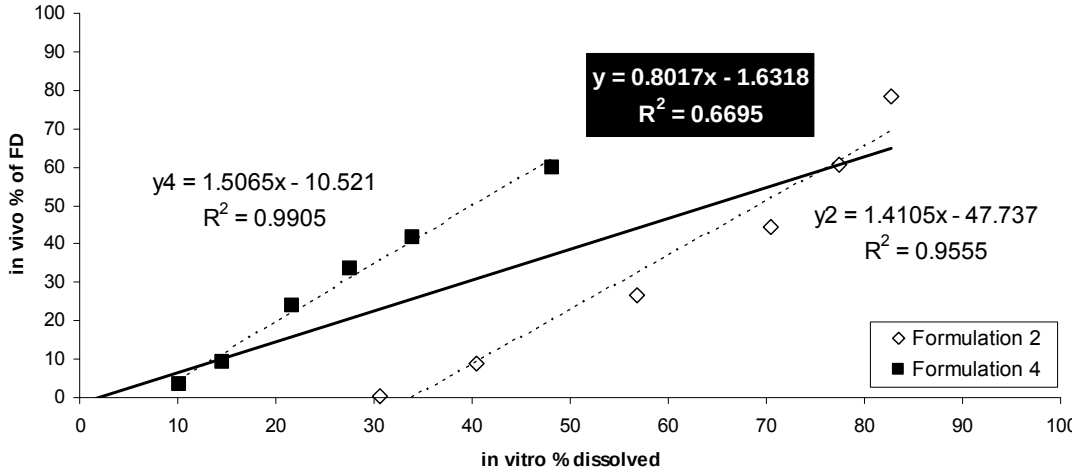


Figure 6 : Logarithm scale of Wagner Nelson.

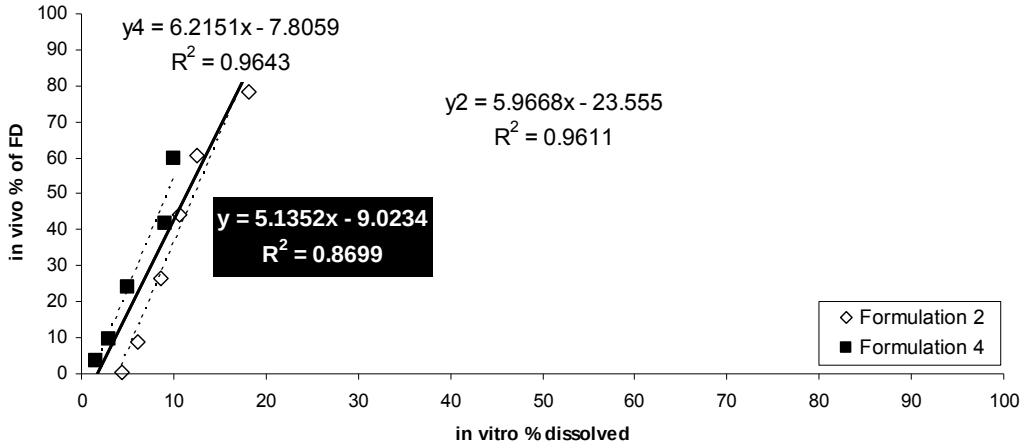
Hcl 0.1N


y = 0.885x - 16.278
R2 = 0.899
y4= 0.9599x - 13.473
R2 = 0.998
y2 = 1.0506x - 33.32
R2 = 0.969
formulation 2
formulation 4
in vivo % of FD
In vitro % dissolved

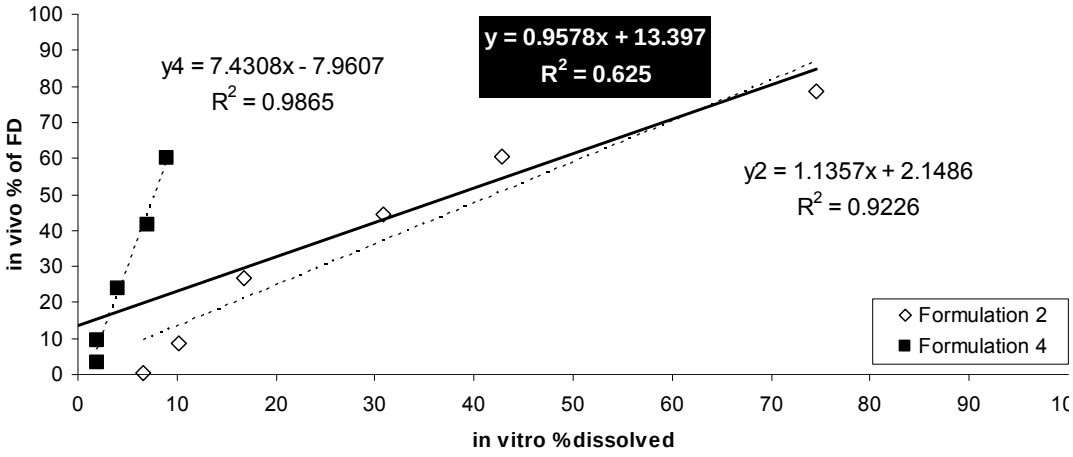
pH 6.0


y = 0.8017x - 1.6318
R2 = 0.6695
y4 = 1.5065x - 10.521
R2 = 0.9905
y2 = 1.4105x - 47.737
R2 = 0.9555
Formulation 2
Formulation 4
in vivo % of FD
in vitro % dissolved

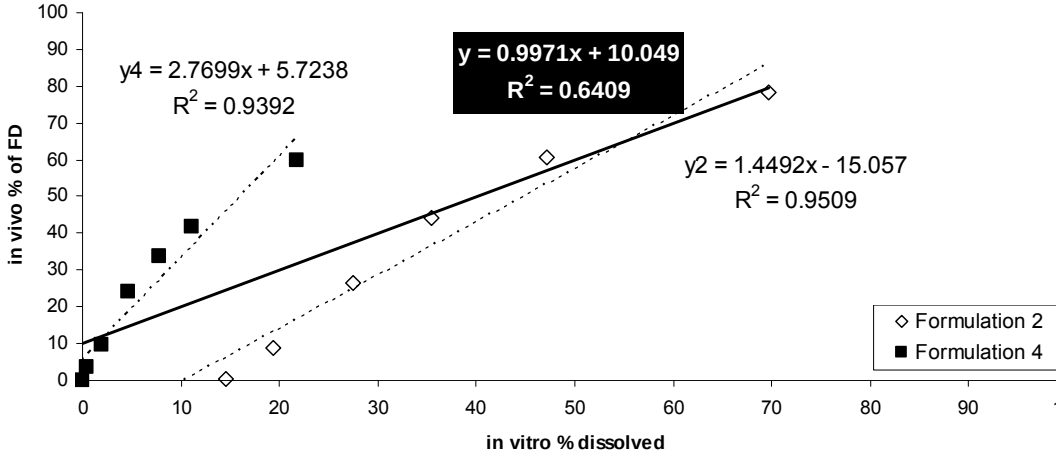
FaSSIF



FeSSIF



pH 6.0 + CTAB 1% (m/v)



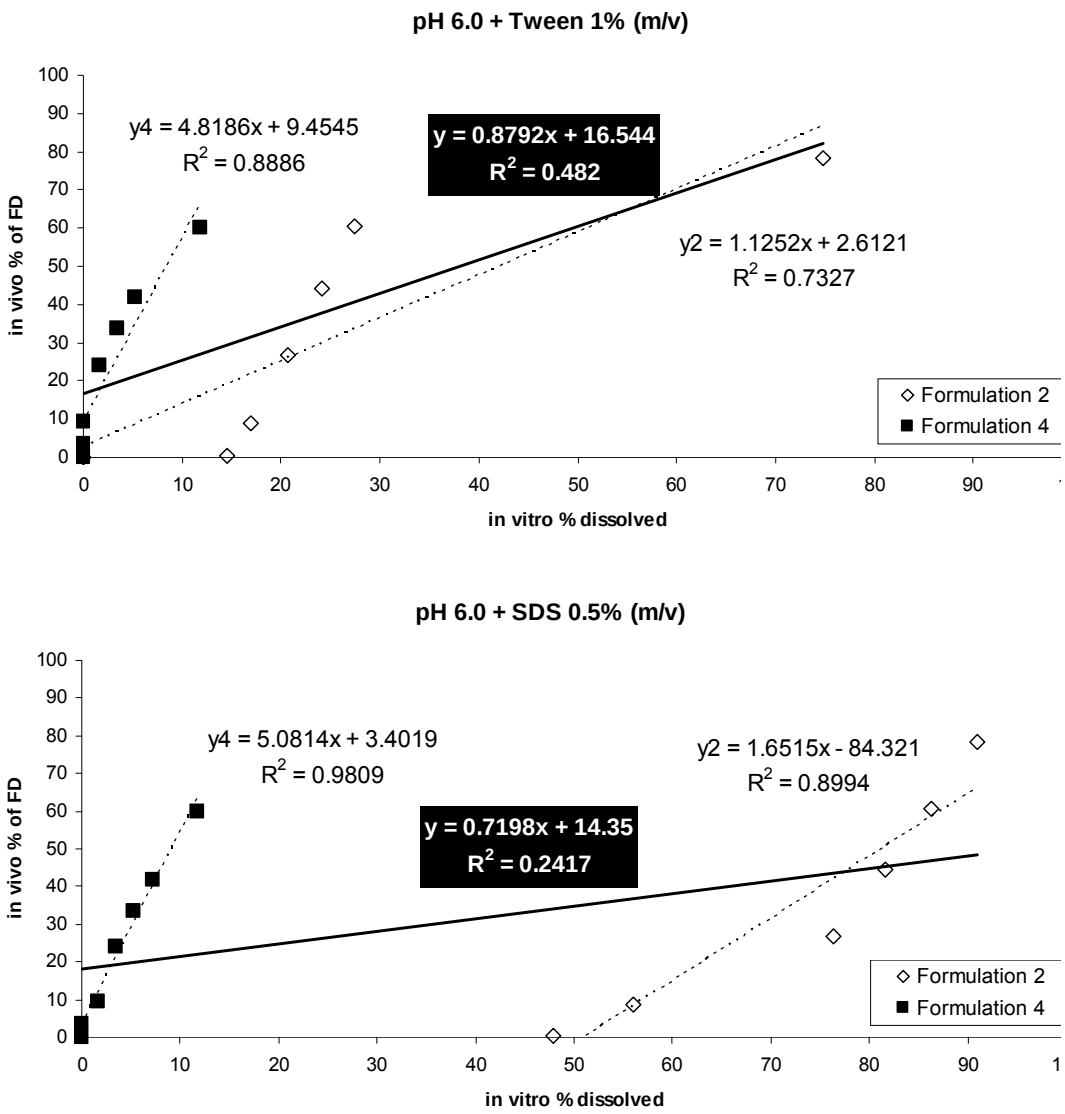


Figure 7: IVIVC attempt for both formulations N° 2 and 4 in different media: a) HCl 0.1N, b) Buffer pH 6.0, c) FaSSIF, d) FeSSIF, e) Buffer pH 6.0 with CTAB 1% (m/v) , f) Buffer pH 6.0 with Tween1% (m/v), g) Buffer pH 6.0 with SDS 0.5% (m/v). Full line indicated the overall correlation for formulations N°2 and N°4, Open symbols indicated formulation N°2 and full symbols formulation N°4. Corresponding equations are identified with y2 and y4 and y for overall correlations.

Discussion

The IR formulation exhibited a fast absorption (Tmax = 1 hour) and a high peak followed by a biphasic decline denoting a two compartment model. This biphasic decline was not observed for both ER formulations confirming, as they behaved as a one compartment model, the possibility to use Wagner Nelson equation.

For ER formulations, the observed Tmax are close to the forecasted time of release. It had to be noted that the lack of sampling time points between 7 and 24 hours, due to limited blood volumes permitted in monkey, might underestimate of the Tmax value of formulation N°4 as well as Cmax and AUC. AUC decreased when absorption was slower (lower Cmax and increased Tmax). The decrease of AUC was, as a mean; lower than the Cmax decrease but of a similar magnitude. For formulation 2 and 4, the diminution of Cmax was respectively 48% and 91% and of 38% and 85% for AUC. The GI transit time of the monkey being shorter than in man (Masayuki and all 2008), incomplete absorption due to the slow release was anticipated, in particular for the formulation 4. Due to the longer residence time in man, the decrease in exposure is expected to be lower. If high initial Cmax values are related to the observed clinical side effects, then the modified release formulation meets the target and the technical feasibility and efficacy is validated.

7 different dissolution media at various pH from HCl 0.1N pH 1.1 to phosphate buffers at pH 6.0; from a fasting state simulated intestinal fluid without lipid components (FaSSIF) of pH 6.5 to a fed state simulated intestinal fluid (FeSSIF) of pH 5.0, were employed on the dissolution USP 2 apparatus. All the media studied were capable to some extent of differentiating between both formulations and the expected rank order was found for the formulation 2 and 4 (ER 4H and ER 8H) respectively. Despite the fact that based on the measured solubility, the entire drug should be freely dissolved into the volume tested; huge differences in dissolution rate as well as very poor recovery were found for some profiles. Profiles obtained in HCl and phosphate pH 6.0 exhibited the highest performance for both formulations. The dissolution profiles are faster than all the others in HCl 0.1 N (pH 1.1). Since HPMC is a polymer with a release behavior independent of the pH (Royce, Li et al. 2004) and since sink condition can be easily reached at pH 1.1 (see table 1) the phenomenon observed in dissolution was the sole reflect of the influence of the excipients on the release of the drug. The improvement of the apparent solubility at pH= 6.0 by addition of surfactant of either nature did not improve the dissolution

and led to worst results than those observed at pH=6.0. Surprisingly good dissolution up to 80% were observed in phosphate buffer at pH 6.0 (at pH 6.0 sink condition was not reached). The good pH=6.0 results were not expected since the drug is a weak base with pKa of 4.6 and solubility decreases with increasing pH. The increase in solubility between FaSSIF and FeSSIF was only reflected slightly on the dissolution of the formulation 2. By contrast no significant difference can be observed for the formulation 4. The release in FaSSIF medium is much slower than in any of the other media examined. This is not expected in view of the high solubility measured in this medium but can be explained to some extent by the low buffer capacity of such a medium as already reported in the literature (Corrigan, Devlin et al. 2003). It appears to be clear that using biorelevant media and medium containing surfactant, the release mechanisms which generally control hydrophilic matrix tablets by diffusion, swelling and/or erosion (Royce, Li et al. 2004; Conti, Maggi et al. 2007) are strongly impacted depending on the HPMC ratio used in the ER formulations. Therefore the observed in vitro performances seem not controlled by the solubility of the API, but rather by the release mechanism. The RO-X molecule behaves in this environment presumably more likely as a BCS class I than like a classical weak base, BCS class II. The dissolution limiting step being controlled solely by the formulation (Costa and Sousa Lobo 2001; Sriamornsak, Thirawong et al. 2007).

By applying IVIVC, in vitro release profiles were compared to the corresponding in vivo input profiles. For most of the media a linear response (R^2 close to 1) for solely formulation 2 or formulation 4 (ER4H or ER8H) can be achieved (see table 3a). Depending on the medium used, similar slopes for ER4H or ER8H can be observed (see table 3b). The use of various in vitro working conditions improved differentiation between formulations but did not necessarily lead to an acceptable and useful correlation with in vivo absorption rates having both formulations 2 and 4 fitted simultaneously. The systematic shift of the correlation shape underlines the sensitivity of the IVIV relationship to medium composition and release mechanism.

Since the release mechanism changes depending on the medium used (but should be similar within the same medium for both formulations) the observed difference in slope can highlight that the dissolution rate is different between in vivo and in vitro, that indicate rather a non suitable dissolution.

In case of strong positive intercept (table 3c) the relationship is considered as not of a good quality as a certain percent is suppose to be absorbed when no part is dissolved in vitro (e.g. in medium with surfactant). Similarly a slope markedly greater than one could indicate that a great part (even 100%) is absorbed when only a small fraction is dissolved (e.g. FaSSIF). In case of a slope lower than one a time scaling factor (e.g. Levy plotting) could be investigated but since the low percentage dissolved observed should correlate to a high quantity absorbed, the conclusion would be that the dissolution test did not adequately reflect the in vivo behavior. Thus, it is obvious that the observed relationships denote that some media are not adequate to perform IVIVC. The correlations resulting from the media containing surfactant are the most weak, and might be due to interaction between the surfactants and the excipients leading to similar in vitro dissolutions, hiding differences in release, even if differences between the release rates existed in vivo. Both ER formulations exhibited similar apparent absorption mechanism (see figure 6) the first choice for the level A correlation focused on the media where each tested formulation resulted as well in a similar behavior with regard to release rate. The aim was to obtain the simplest model possible. With this consideration in mind, the only apparent IVIVC which showed similar drug release mechanism, which were linear and resulted in similar slope for both ER variants were obtained using FaSSIF, pH 6.0 buffer and HCl 0.1N. For those 3 media, release did not start at a similar time; formulation 2 is faster than 4, that being in line with the expectations. However for FaSSIF, the slope is largely greater than 1 indicating that for a small amount dissolved (e.g: only 20% dissolved after 7h for the formulation 2), a large amount was absorbed leading to the conclusion that this media is not optimal. For pH 6.0 buffer a great difference in intercept was observed for the formulation 2 compared to formulation 4, leading to a poor overall coefficient of correlation (R^2 =0.67). The

negative intercept was always longer for formulation N° 2 compared to formulation N° 4 (Table 3c) indicating that in vitro the dissolution was slightly faster than the absorption for formulation 2 compare to 4. The best level A correlation observed was obtained using HCl 0.1N. The overall linear regression yielded a regression coefficient R^2 of 0.90.

By applying the IVIVC equation obtained with HCL, a prediction according to inverse of WN method (Gohel, 2005) and based on the observed in vitro dissolution was tried and the results are presented in figure 8.

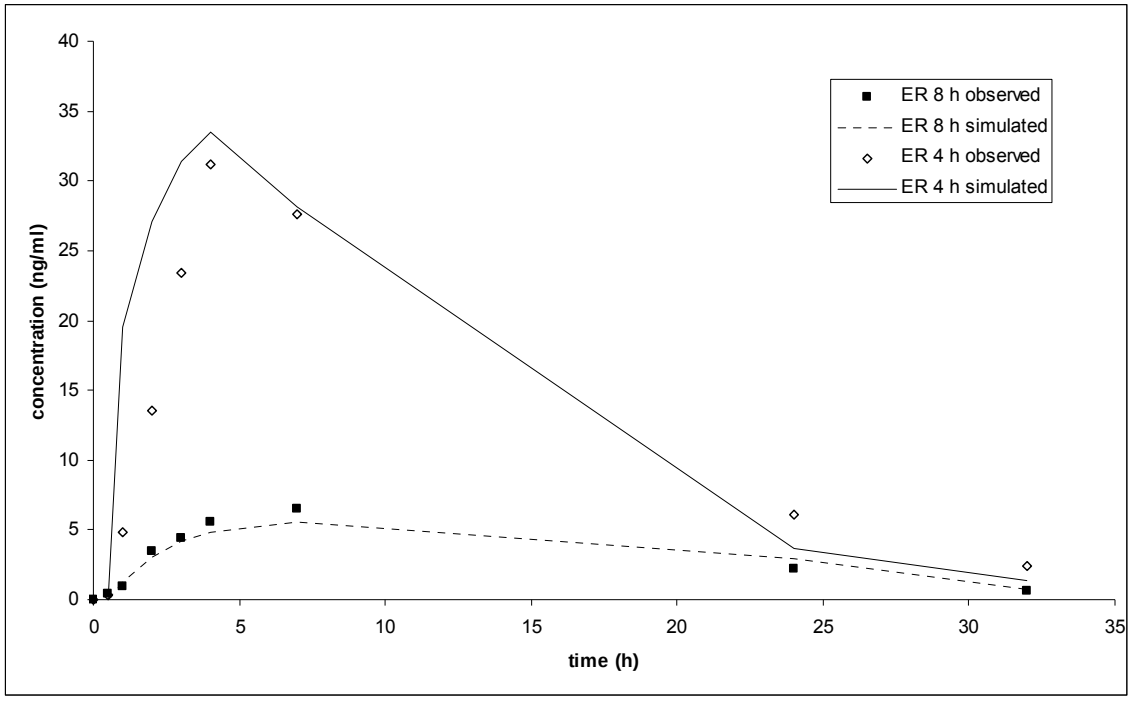


Figure: 8 Prediction of absorbance profiles based on HCl 0.1N dissolution data and IVIVC.

The prediction resulted in an error of +13% and -7% on Cmax for ER 4H and ER 8H respectively and on a negligible error on AUC and Tmax (less than 1%) denoting a rather good predictability. Based on this correlation, dissolution in HCl 0.1N medium can adequately support the development and improvement of further formulation.

Among the methods in the literature to ascertain the kinetic modeling of drug release, e.g: zero-order, first-order (Gibaldi and Feldman 1967), Higuchi (Higuchi 1963), Hixon-Crowell

(Hixson and Crowell 1931) and Weibull models (Langenbucher 1972; Papadopoulou, Kosmidis et al. 2006) the exponential equation of Korsemeyer and Peppas (Peppas 1985), as well as Harland (Harland, Gazzaniga et al. 1988; Kim and Fassihi 1997; Kim and Fassihi 1997) are used to describe the drug release behavior from polymeric systems. These models are generally used to analyze the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than one type of release phenomenon could be involved. As Korsemeyer and Peppas equation can only be used to fit 60 % release, the best fit was obtained by using Harland et al. Thus the dissolution profiles can be fitted to the equation (1):

$$Mt/M\infty = A\sqrt{t} + Bt \qquad \text{eq. (1)}$$

In the above equation, A and B are diffusion and erosion terms. When A>B, the diffusion factor prevails in the release system. When A<B, erosion predominates. If A=B, the release mechanism includes both diffusion and erosion equally (Ratsimbazafy, Bourret et al. 1996).

The dissolution profiles in HCL fitted by eq. (1) suggested clearly an apparent diffusion mechanism associated to the in vitro release for both formulations (ER4H A = 0.38; B=0 and ER 8H: A = 0.25; B= 0.02) and confirmed that both formulations behave similarly in this medium. Controlling of the diffusion behavior can then be an additional monitoring parameter to support new formulations or change during further development.

In comparison to more complex approaches using simulated intestinal fluids as often seen in literature reports (Dressman, Amidon et al. 1998; Jantratid, Janssen et al. 2008; Lu 2008) the use of this simple dissolution method is advantageous since it has the potential to serve both as a robust quality control method and a biorelevant method with discrimination power.

Attention has to be paid on the fact that the present observed correlation is valid only for an apparent one compartment model in vivo absorption using a hydrophilic matrix.

Overestimation of these criteria can lead to misinterpretation even if the mathematical correlation seems to suggest a reliable prediction of the in vivo performance (Dokoumetzidis 2008; Rettig and Mysicka 2008).

Additionally it is important at this stage of the development to note, that this animal approach using IVIVC, has a number of limitations the major one being the gastrointestinal capacity and transit time of monkey compared to humans. Monkeys are closer to humans in terms of evolutionary development than all other commonly used laboratory animals like rodents or dog (Kalarli 1995). As a model for oral drug absorption, cynomolgus monkeys can be used to address intra and inter individual physiological variability (Willmann, Edginton et al. 2007). Apart from species differences in intestinal physiology, erroneous assumptions can also be made with regards to scaling of dosage. In our case, the drug was tested in animals as side effects were observed in humans and a new in vivo study in humans could not be proposed before the safety of the drug was assessed in animals. Rodents cannot be used due to size of tablet, dog was not possible due to constriction force of the stomach that is known to destroy HPMC matrix (Kamba, Seta et al. 2002), and therefore the monkey was the only practicable species. The large variability observed between animals, implied difficulties to predict small variations and limit the discriminative power the IVIVC linked to minor changes of the formulation. However the aim of the present work was to be able to discriminate formulations with a large release difference in order to insure the best selection before Human study and to check which dissolution test was the most appropriate that being done based on the mean curve which is an unbiased estimation.

In silico estimation was evaluated as well but also exhibited some limitations. Nowadays, several computational simulation programs are available (GastroPlus™; WinNonLin®; Parrott and Lave 2002) and are offering specific modules (e.g. IVIVC Toolkit). However, computational simulation is not always accurate since it is based on many assumptions like numeric integration of the Noyes-Whitney equation for the dissolution or the membrane permeation equation. Incorporation in the simulation of factors like the relationship between

disintegration, dissolution and erosion/diffusion mechanism or furthermore the increase of solubility due to addition surfactant but leading to slower dissolution as observed within this investigation can not be easily set up using the current version of these software's (Kesisoglou and Wu 2008; Okazaki, Mano et al. 2008). In most of the cases a model can only be adequately setup after a first comparison with real experimental data and some adjustments (Sugano 2007). Thus until these criteria can be adequately modeled, generation of the appropriate in vitro data to try to approximate the in vivo behavior and use as input to establish a correlation will remain more accurate. However, the data generated within this study to demonstrate its technical feasibility for the ER formulation, enable now a better basis for further in silico projection, in particular for simulating human behavior based on animal data.

For drugs like RO-X associated as low dose with hydrophilic matrixes, first attempts can be done in classical acidic media (pH 1.1 to 2.5) and neutral media (pH 5.5 to 7.0). In case of similar and conclusive results between the two dissolution tests, the animal models can be used to confirm the ranking of the formulations and the expected results. In this case an IVIVC/R investigation can be much easily set up. As general rule for IVIVC investigation, the correlation is more realistic if the release mechanism and rate observed in vivo is reflected in vitro. In vitro working condition that can be adapted consequently in case of poor relationship. The information likely to be gained is worthwhile and in this way, the efforts to achieve a correlation, facilitate formulation screening at the early development stage by better understanding of key parameter that are likely to impact the drug product performance. Additionally the use of an IVIVC based on animal species in early stage potentially reduce the number of animal studies, that are typically done for formulation screening, that being in line with current recommendations. Anyhow these investigations provide valuable information to better streamline the drug development process and offer help in evaluating manufacturing process parameters at later stages.

Conclusion

The in vitro release from the developed formulations was found to be independent of solubility and pH but dependent mainly of the composition of the dissolution media. This investigation shows that the simple HCl medium was superior to a biorelevant medium and medium containing surfactant when investigating the drug in cynomolgus monkey and establishing IVIVC. The results of the dissolution in HCL have helped to identify the diffusion mechanism, from a HPMC based extended release formulation, as the apparent key parameter of the release mechanism.

The significance of the present study may be applicable to other ionizable weak base drugs with high permeability. For this BCS class II compound (weak base, low drug load), the dissolution rate of this extended release form is not limited by the solubility over GI tract pH but mostly by the release mechanism. During early drug development, it is extremely useful to have a predictive in vitro dissolution test that correlates with in vivo absorption. Such a test helps in screening of new formulations as well as evaluating changes in existing formulations with regard to their impact on bioavailability (BA). In this case further research has to be conducted to confirm this outcome in man. In conclusion, adjusting dissolution testing conditions to match the behavior of the formulations in vitro with that in vivo is a simple and useful approach in identifying a predictive method in development of in vitro-in vivo correlation and allows clearly decreasing the risk before first entry into human.

Acknowledgment

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Declaration of Interest

The authors report no declaration of interest.

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Selection of the Most Suitable Dissolution Method for an Extended Release Formulation based on IVIVC level A obtained on Cynomolgus Monkey

Supplement 1: Confirmation of IVIVC on human

Introduction

In order to confirm the outcome of the Proof of Mechanism observed on monkey for both ER4H and ER8h formulations, a BA study was initiated in human as PoC.

The Ro-x Molecule is currently in phase II development at Roche and several studies are on going. Due to some IP limitation, only reduced data set of the clinical results of comparison immediate versus modified release are presented in this supplement of the paper. The aim of this investigation was to assess if the correlation found on monkey can be further used on human. The same tablets composition was used, allowing to directly comparing the data. The combination of dissolution, in vivo data and correlation is discussed in the present supplement as a smart development tool to speed up the realization of new formulations and to ensure the best performance during the further development of the formulation.

Material and Method

Both ER4H and ER8H 1 mg tablets tested on Cynomolgus monkey were further used for a PK human study.

In vivo bioavailability assessment

A single-center, double-blind, randomized, three-period cross-over study with 12 healthy volunteers was performed. The IR release 1 mg formulation was compared to the ER4H and ER8H in fasted state.

The samples were collected at predefined time intervals (0, 0.5, 1, 1.5,2, 4, 5, 6, 8, 9, 10, 11, 12 and 24 hours) and measured by a validated HPLC-MS method.

In Vivo-In Vitro Correlation

For the in vivo data, in addition to the classical bioavailability parameters Cmax, Tmax, AUC, the percentage of fraction of drug absorbed (% FD) was determined by numerical deconvolution using the software Gastro plus© with IVIVC toolkit and a Weibull function. In vitro the percent of drug dissolved (% D) were obtained from the dissolution mentioned in the paper 2. The in vivo and in vitro data were put in relation using a point-to-point relationship between the in vitro dissolution and the in vivo input of the drug (IVIVC Level A). Linear regressions were primary sought. Calculations were performed with Microsoft Excel©.

Results

In vivo-data and in vitro data

The in vivo results are presented in figure 1 for PK parameters. For confidentiality reason detailed information are not presented.

The IR formulation exhibited a fast absorption (Tmax = 1 hour) and a high peak followed by a biphasic decline denoting a two compartment model. This biphasic decline was not observed for both ER formulations, confirming that was observed on monkey. This observation is well in line with observations made on figure 4 of paper 2.

The in vivo release data demonstrated that compared to the IR reference a lower Cmax and a prolonged Tmax can be observed with both extended release (ER) tablets indicating a slower absorption and an impact of the composition of the tablets on the global performance. The data after deconvolution are presented Figure 2. After 4 hours the ER4H showed an apparent absorption of 75 %, 100 % release being reached after approx 7 hours. After 8 hours the ER8h exhibited an apparent absorption of 70%, 100 % release being reached after approx 24 hours. The corresponding in vitro data for the ER4H and ER8H are presented Figure 3.

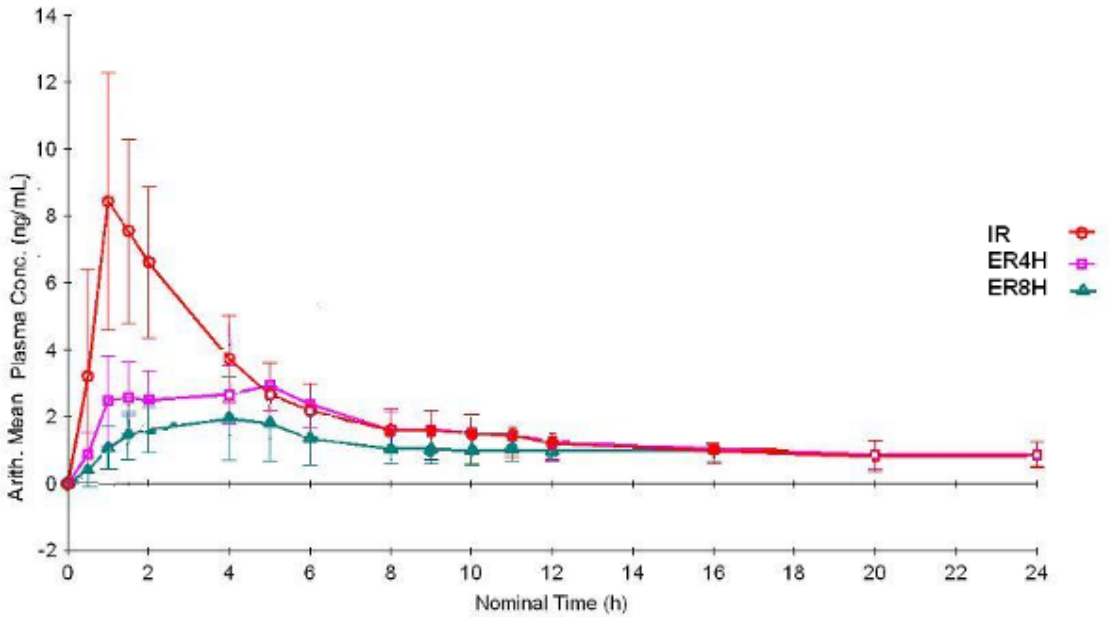

Figure 1: PK mean profiles of RO-x in human for the 3 tested formulations (ER4H and ER8H versus reference IR formulation).

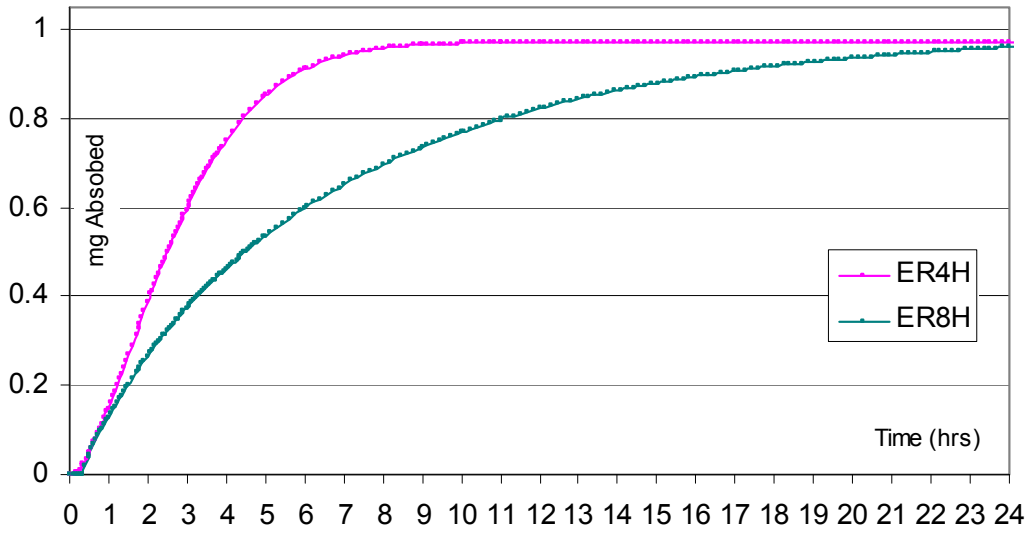

Figure 2: mg of fraction absorbed in function of time for ER 4H and ER8H after deconvolution.

IVIVC

The in vitro–in vivo correlation level A attempted is presented in Figure 4 and in Table 1.

A linear response (R^2 close to 1) for solely ER4H and ER8H as well as both fitted together can be achieved. Similar slopes for ER4H or ER8H can be observed. The negative intercept indicating that in vitro the dissolution was slightly faster than the absorption for both formulations. It can be seen from Table 1 that similar data to those observed on monkey have been found.

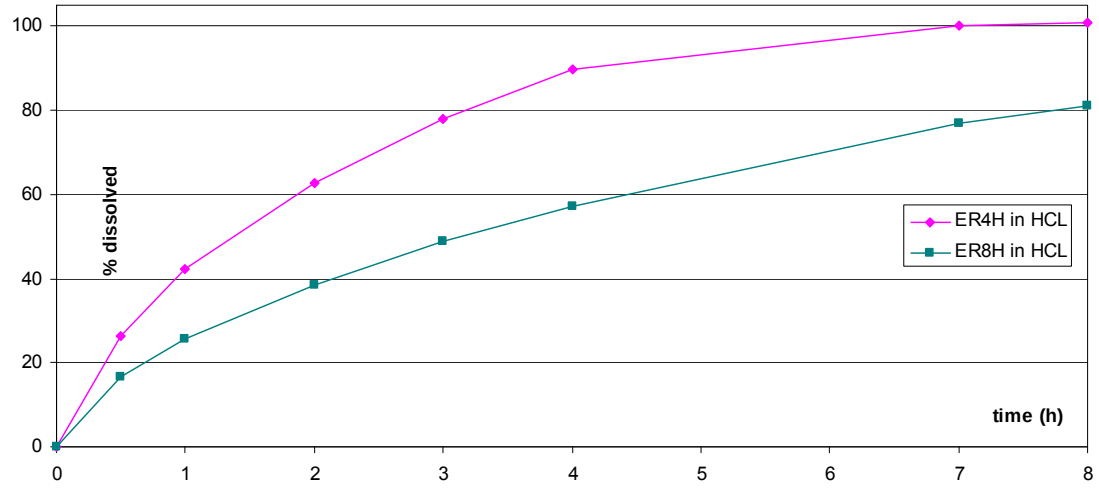

Figure 3 : In vitro Dissolution Profiles of ER4H and ER8H in HCL 0.1N

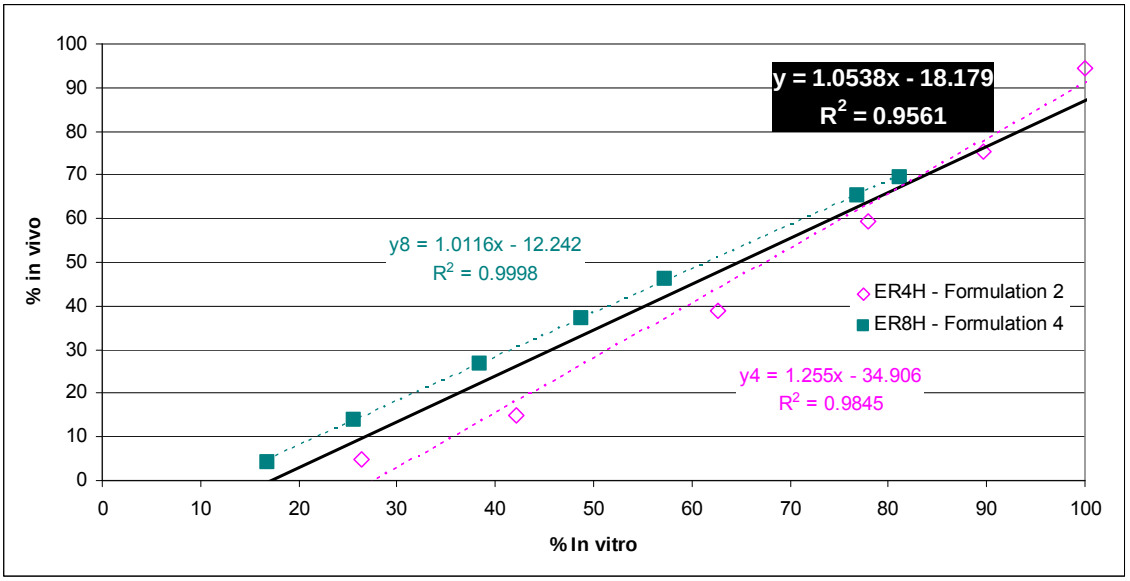

Figure 4: IVIVC level A on human data for ER4H and ER8H in HCl. Full line indicated the overall correlation for ER4H and ER8H. Corresponding equations are identified with the different colors and y4 for ER4H y8 for ER8H and y for both fitted together.

Table 1: Overview of the obtained parameter for IVIVC level A

	R^2	*slope*	*intercept*
ER4H	0.98	1.01	-12.24
ER8H	0.99	1.26	-34.91
ER4H +ER8H	0.96	1.05	-18.18

Discussion

The prediction of the extended releases performance assessed on monkey has been confirmed on human. The figure 4 here is well in line with the figure 7 (HCL 0.1N) of paper 2. Those findings indicated that any relation established in monkeys could be, as an initial step, extrapolated to human for this compound in order to faster the development of the final formulation. Furthermore the in vitro assessment can serve as surrogate for the in vivo performance during development.

Thus a simple and cost effective media was shown to be potential substitutes for the more complex, physiologically based Fa/FeSSIF. It will not only be economic during the various steps of drug development, but could also place the quality control dissolution tests into a more meaningful context.

Frequently, drug development requires changes in formulations due to a variety of reasons, such as unexpected problems in stability, availability of better materials, better processing results, scale up issue etc. Having an established IVIVC can help avoid bioequivalence studies by using the dissolution profile from the changed formulation, and subsequently predicting the

in vivo concentration-time profile. This predicted profile could act as a surrogate of the in vivo bioequivalence study.

Furthermore, IVIVC can allow setting and validating of more meaningful dissolution method and specifications. It can also assist in quality control for certain scale-up and post approval changes. It is useable as a strong derisking tool.

Both the regulatory agencies and pharmaceutical industries have therefore understood this value of IVIVCs. It reduces the need of human studies and has enormous cost-saving benefit in the form of reduced drug development spending and speedy implementation of post-approval changes. The nature of post-approval changes could range from minor (such as a change in non release-controlling excipient) to major (such as site change, equipment change, or change in method of manufacture, etc) (FDA, Supac MR 1997).

Conclusion

This example showed perfectly the role that could play dissolution in the elaboration of a discriminative test to surrogate bio performance.

The importance of a representative dissolution testing method that accurately describes the in vivo release rate is well highlighted in the paper 2 and this supplement.

The role of the dissolution testing method in IVIVC development and validation is to serve as a surrogate measure of the rate and extent of oral absorption.

A valid IVIVC will allow for dissolution testing for subsequent formulation changes which take place as a function of product optimization without the need for additional bioavailability /bioequivalency studies.

Such a test helps in screening of new formulations as well as evaluating changes in existing formulations with regard to their impact on bioavailability .

Selection of the Most Suitable Dissolution Method for an Extended Release Formulation based on IVIVC level A obtained on Cynomolgus Monkey

Supplement 2: Understanding of the release mechanism using imaging technology.

Introduction

During the investigation of the release mechanism of the ER4H and ER8H HPMC ER formulations , the diffusion was mathematically identified as the main mechanism that is likely to impact the performance (see paper 2).

When a hydrophilic matrix is exposed to water or a biological fluid, it starts to hydrate and swell from the outer boundary towards the center. A gel layer is formed around the matrix, which significantly influences the dissolution and diffusion of the drug through the polymer. Various authors have investigated the phenomena involved in the swelling and drug release process for systems containing HPMC. They have concluded that the polymeric content, which is related to swelling behavior, and the viscosity grade are the determining factors in predicting the drug release from hydrophilic matrices (Ranga-Rao 1990, Atzhendler 2000). Lee and Peppas (Lee 1987) defined the boundary between the matrix surface and the dissolution medium, as the ‘‘erosion front’’ and the boundary between the glassy polymer and its rubbery gel state as the ‘‘swelling front’’ (figure 1). Additionally the presence of a third front within the gel layer was observed in a matrix containing sodium diclofenac as the active substance. This was identified as the ‘‘diffusion front’’ and its boundaries are within the gel layer, between the areas in which the drug has dissolved and not dissolved (figure 1) (Lee 1987). Further, it has been demonstrated that the behavior of the diffusion front depends on the solubility and the quantity (or loading) of the active substance (Bettini 1998). These three fronts can be observed and their movements facilitate the calculation of the parameters of the swelling/dissolution process. In fact, a synchronization of the movements of both the swelling and the diffusion fronts is the essential step to achieve a constant drug delivery rate.

To better visualize this gel formation imaging technologies can be used. Several methods have been described in the literature in the last decade for the study of the gel layer. These include for instance the NMR imaging, (Shapiro 1996) or ultrasound (Rajabi-Siahboomi 1994) methods together with optical methods, such as image analysis (Sung 1996) or more recently Conti et al (Conti 2007) used image analyzer (digital microscope camera) for morphological

studies. Since each technique is applied under different experimental conditions, it is difficult to determine whether one is superior to the others.

In this complementary paper, a X-Ray Computed Microtomography (XCMT) imaging system has been used to observed the gel layer formation. This method measures both the dimensional changes of a matrix tablet and the gel layer growth in the tablet during swelling. This can be observed directly into the tablet since this technique offers a non invasive qualitative and quantitative tool to study in 3 dimensions (3D) swelling and solvent transport within the matrices.

The varying levels of signal intensity of the XCMT provide a gray-scale in the images from which information about the density, thickness, and attenuation properties of the sample can be obtained. Very dense or thick regions and areas that contain heavy elements (e.g., sodium, chlorine, or iron) will generally create the most contrast in the final images. In very simple terms, X-ray microtomography can be thought of as creating a three-dimensional map of the relative atomic density of the sample under evaluation. A virtual cut of the tablet is performed and the different density observed.

It is important to note, that this approach was very experimental. Due to the limited amount of material available and the early stage of the formulation at the time of the investigation, only limited investigation can have been done. Very few references were found in the literature associating this both techniques (Sinka 2004, Busignies 2006, Zeitler and Gladden 2008, Laity 2010) and several dry runs were necessary to propose valid measurements.

Nevertheless the limited amount of data presented were generated in order to highlight the potential of a very innovative and promising imaging technique associated with dissolution to assess the performance of a formulation.

In this complementary paper, the swelling behavior of the ER4H tablets versus the dissolution time was investigated using this imaging technology. The observations gained with imaging are correlated with the dissolution and are discussed as valuable information in view of process understanding and identification of CQA.

Materials and Methods

Extended Release tablets and morphological studies using imaging

Both ER4H and ER8H 1 mg tablets tested in cynomolgus monkey and in human were investigated.

The in vitro dissolution experiments performed in HCL, as described in paper 2, was repeated. To analyze the morphological behavior of the tablets during the release process, tablets were withdrawn from the dissolution vessel at different time intervals. Samples were collected at 15, 45, 90, 120, 180, 240 minutes. Due to the low amount of material available, only one tablets was measured at each time points. The analyses were repeated twice.

The tablets were measured intact, wetted. The XCMT scans were obtained using a Skyscan 1172 high-resolution desktop XCMT system (Skyscan, Aartselaar, Belgium) with a 100-kV X-ray source tuned to 60kV -167µA, with a rotation increment of 0.4 degree over a 180° rotation. Camera resolution was 2000x1200 pixels at 8 µm per pixel with a scan time of ca. 13 min. The acquired shadow images were processed via a cluster of four servers in order to obtain the virtual projection images. The softwares DataViewer and CTVox (Skyscan, Be) were used for the visualization of the stack of projection images and the 3 dimensional display of the object respectively.

Basically, X-Ray Computed Microtomography (XCMT) uses a mathematical algorithm to reconstruct a three-dimensional structure from multiple two-dimensional X-ray shadow images. The sample is placed on a precision turntable between a high power X-ray source and a detector array (line or area array), which is used to measure the intensities of the diverging X-ray beam transmitted through the sample (Sinka 2004, Busignies 2006). Multiple attenuation coefficient values, which correlate to the degree of attenuation of the X-rays, are obtained as the sample is rotated relative to the X-ray beam (to obtain multiple sets of attenuation coefficients from different viewing angles). This raw data can be converted to pixel greyscale data, which a mathematical algorithm can translate into two-dimensional grey-scale radiograph or projection images. Furthermore, the computer system can be calibrated so that values are assigned to certain materials. For example, air may be assigned a value of zero (black) and water a value of one thousand (white) allowing the pixels to correspond to variation in density. A three-dimensional reconstruction, called a tomogram, is formed from this set of two-dimensional images using a mathematical algorithm that is based on the Beer–Lambert law of absorption. The digital unit for this picture is called a voxel. Similar to pixels, voxels can be calibrated to display apparent density.

In addition to the morphology evaluated towards the analyses of the pictures, the size and volume of the tablets at the different time points can be assessed semi quantitatively (data not shown).

Results

The X-Ray micro Computed Tomography allows measuring the entire tablets after withdrawal out of the dissolution vessel. An example of the 3D image collected is shown figure 2. The gel layer appears as a brighter zone. Undissolved drug in the glassy polymer layer appears darker. To better visualize the two boundaries of the gel (swelling front and erosion front) a virtual cut of the tablets from the middle on the larger side was performed. The 2D images are presented figure 3. The swelling/diffusion front is qualitatively indicated in figure 3 by an orange ring. The pictures presented figure 3 have all the same scale.

From visual observation it is clear that as soon as the tablets are exposed to the dissolution medium, the liquid penetrates into the matrix and the hydrated polymer swells to form a gelatinous layer around the tablet. From 15 minutes to 120 minutes a clear change in the morphology of the tablets can be observed. Modification of the form of the tablets as well as the size and contrast of the diffusion front (highlighted with the orange ring) are obvious.

In fact, upon contact with the dissolution fluid, the tablets hydrate slowly and swells showing a thick gel layer after 15 minutes. The gel layer and a partially wetted core can be clearly identified. The gel thickness increases progressively moving inwards as a function of hydration, the dimensions of the solid core decrease. Only a small non-gelled core is visible after 120 minutes and after 180 minutes the tablet appears to be completely gelled. After 180 minutes, the matrix appears completely hydrated and gelled (it is not possible to see the presence of any glassy core).

Please note that the black holes that can be seen in some pictures are artifact due to the manipulation of the tablets during the extraction out of the dissolution vessel using a pliers.

Change of composition during dissolution by observation of a diffusion/swelling mechanism can then be visually confirmed (figure 4).

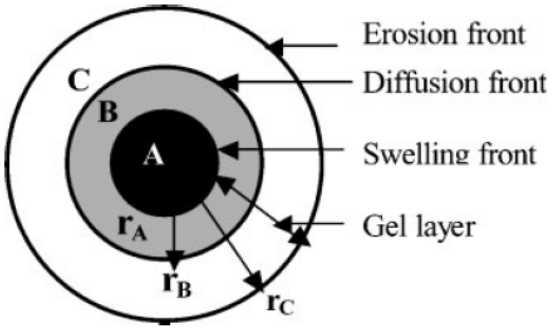

Figure 1: Schematic representation of erosion, diffusion and swelling fronts, along with the relevant layer: (A) undissolved drug in the glassy polymer layer; (B) undissolved drug in the gel layer; (C) dissolved drug in the gel layer, $r_C - r_A$ is the gel layer and r_B-r_A is the diffusion layer. *Figure taken from Lee PI, Peppas NA. Prediction of polymer dissolution in swellable controlled-release systems. J. Contr. Rel. 1987; 6: 207*.

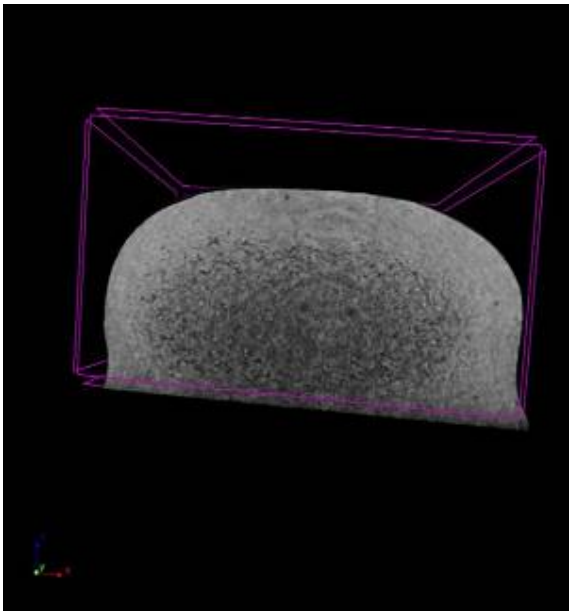

Figure 2: Tomogram (3D imaging reconstructed) of the entire HPMC ER4H tablet after 45 min dissolution in HCl using : X-Ray micro Computed Tomography.

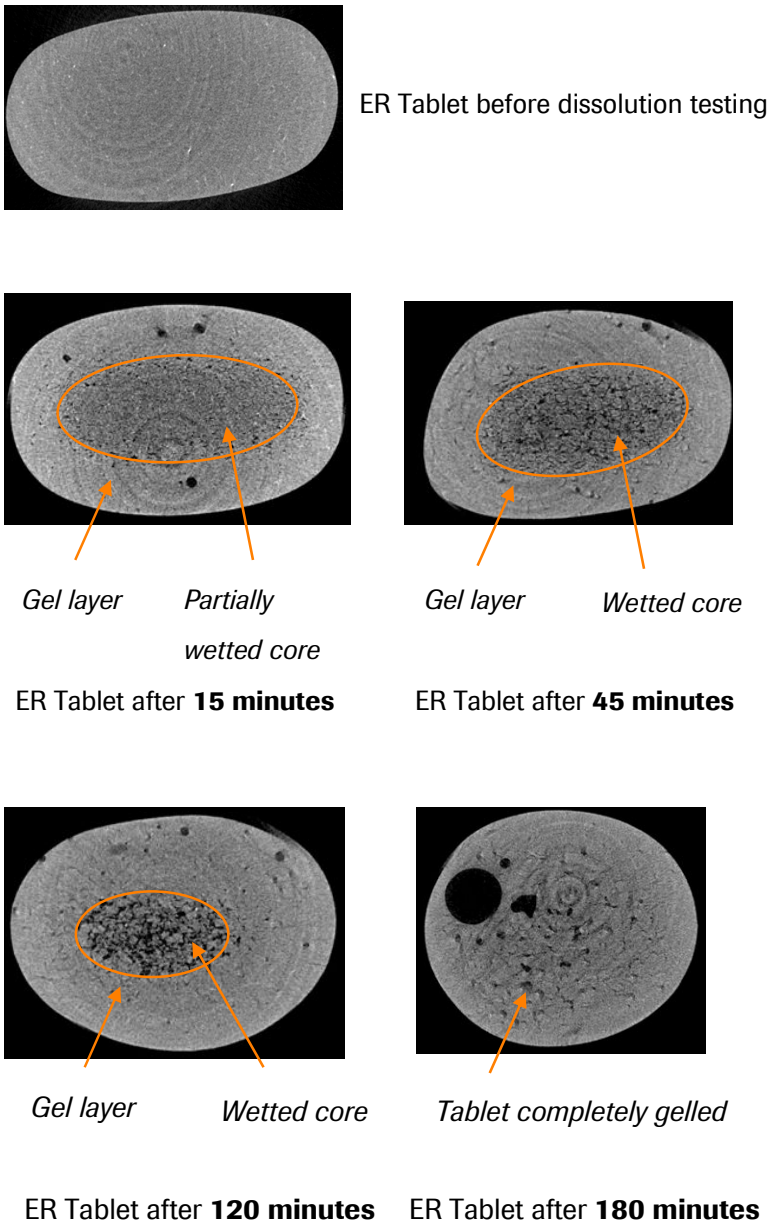

Figure 3: Virtual cut of the tablet at 8 microns resolution X-Ray micro Computed Tomography (X-Ray micro CT) after dissolution in HCL at different time points.

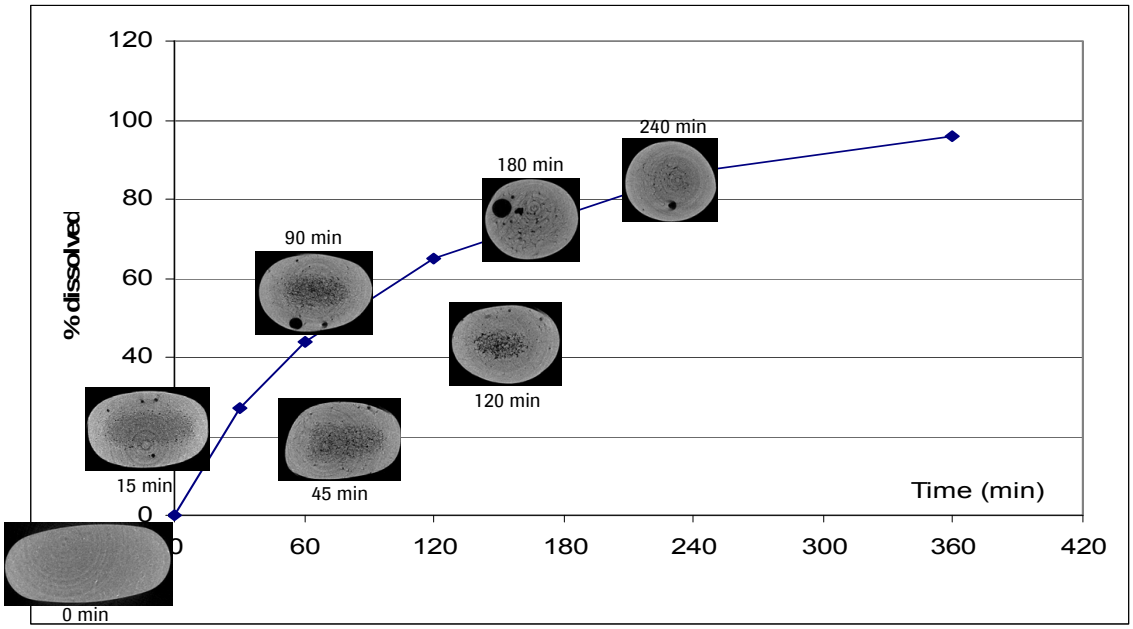

Figure 4: Dissolution profile in HCL monitored with imaging using X-Ray micro Computed Tomography.

Discussion

It is important to note, that this approach was very experimental. Due to the limited amount of material available and the early stage of the formulation at the time of the tests, only limited investigation can have been done. Very few references were found in the literature associating this both techniques and several dry runs were necessary to propose valid measurements.

The results of this morphological analysis have been correlated to the results of the dissolution test with focus on the mechanism of drug release analyzed in the previous work (paper 2). The diffusion mechanism was mathematically identified using the equation from Harland. It should be noted that, while the equation may reflect a simple mathematical relationship, this arises from considerably complex underlying processes.

Based on this limited amount of pictures, the diffusion mechanism can be reasonably confirmed as main phenomenon observed during these investigations based on :

1) After a first wettability phase, the size of the tablets remains nearly constant (visual observation presented here only). With erosion driven mechanism, we should have rather expected, as the HPMC matrix erodes, a diminution of the size of the tablets over the time. This was already observed by Polli, with digital microscope camera (Polli 2007, 2007). More deep investigations may be necessary to confirm it (with thickness measurements for instance).

2) The wetting of the core of the tablets as function of the time can be clearly observed, indicating a swelling of HPMC matrix mainly in axial direction (from surface to core).

This experiment allows tend to confirm the diffusion mechanism with a qualitative analysis.

The aim of this study was to assess the potential of an imaging technology in association with the dissolution behavior. It is primarily devoted to increasing the mechanistic understanding of a formulation and its manufacturing processes, with this regard it is a typical QbD tool. A release mechanism well understood allowing taking a better decision.

Based on the IVIVC presented in the previous papers, the release mechanism associated with the best in vivo performance is the diffusion.
As a new formulation will be proposed (after scale up or excipients quality change for instance), the first assessment will be performed by dissolution.
It is paramount to understand the interrelationship between physicochemical and hydrodynamic conditions in attaining sensitive and reproducible dissolution data.
However, although the dissolution performance (release rate) can be similar; the release mechanisms can be slightly different (from pure diffusive mechanism to non Fickian mechanism for instance). Change of the mechanism can impact the validity of the IVIVC and lead to improper decision.
The evaluation of the release mechanism can be then confirmed with imaging and serve as an additional tool to take the right decision before further testing on animal or human.
It is to note that this technique needs some specific imaging expertise on dissolution and on imaging technology, which highlight the fact to have a strong collaboration between different kinds of science.

Conclusion

Data collected with the novel integrated apparatus were in good agreement with the released mechanism, calculated based on the *in vitro* dissolution test. The limitation of the XMCT here is the off line measurement and the impact of sample preparation on the data. However, this study has shown that XCMT may be a reliable and appropriate method for elucidation of physical transformations that occur during dissolution for HPMC matrix, which to-date have only been studied via theoretical or destructive investigation. In conclusion, XMCT is a sensitive *in vitro* technique suitable for the study of inert matrix tablets with the potential to be used to investigate other solid dosage systems.

The overall goal of either establishing an IVIVC or implementing QbD is to have a better control of the product performance within the life cycle of a product. The use of imaging technology in association with dissolution clearly helps in this way.

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Conclusion experimental 2

Interplay dissolution, in vivo performance on animal, human and identification of CQA were highlighted using this example of ER formulations.
The importance of a representative dissolution testing method that accurately describes the in vivo release rate and allows a clear understanding of the factor acting on the performance, is well highlighted in the paper 2 and both supplements.

Compared with IR product (paper 1) IVIVC is generally more likely for ER dosage forms where drug absorption is normally limited by drug release. To increase the change of success, it is crucial to evaluate IVIVC feasibility, in vitro and in vivo results, by applying integrated knowledge of physico-chemical and biological characteristics of the drug substance, dosage form design and their interplay with the GI tract. It is also important to make an IVIVC strategy an essential part of the dosage form development program.

The relevance of the confirmed data in human performance allows
(1) to cross validate the animal model as suitable model for this compound
(2) to set up the suitable in vitro analytical method(s) to accurately measure the material in quality control.
(3) to set up biorelevant specification.
(4) to identify and confirm the diffusion as main release mechanism likely to impact the performance of these ER formulations
(5) to confirm the importance of the interaction between the different expertise during the development.

3.3. Experimental part 3

To support the development of meaningful dissolution method that allows high discrimination or that aid in IVIVC/R discovery, more complex methods or apparatus than the standard pharmacopeia may be developed.

One way of research can be, for instance, by seeking of medium designed to closely simulate physiological composition to better link in vitro with in vivo performance. However another way of investigation could be to investigate on the hydrodynamics.

In vitro this characteristic can be further challenged by changing the working condition and modifying the apparatus.

The possible use of dissolution in early development phase using non compendia methods has been investigated. The use of small volume vessel and small paddle in place of compendia system is commented using different kind of drug product.

This work is presented as **paper 3** entitled.

"Small Volume Dissolution Testing as Powerful Method during Pharmaceutical Development"

This paper has been published in *Pharmaceutics* in November 2010, Vol. 2, Pages 351-363.

Further investigations using the small vessel and the basket method are presented in the first supplement (paper 3 supplement 1) entitled "**Small Volume Dissolution Testing using Basket method".** The data obtained for the basket are summarized and briefly commented.

On other example of applying small vessel non compendial method is highlighted in the supplement (paper 3 supplement 2) entitled "**Tenoxicam-Methylparaben Cocrystal Formation in Aqueous Suspension Formulation** ", where already in early development during the pre formulation, dissolution can support the cocrystall screening program. This work was presented in the AAPS Annual Meeting 2009, poster W4326.

Article

Small Volume Dissolution Testing as Powerful Method during Pharmaceutical Development

Scheubel Emmanuel [1,2,*] Lindenberg Marc[2] Beyssac Eric [1]Cardot Jean-Michel[1]

[1] Biopharmaceutical department, Faculty of Pharmacy, University of Auvergne 28 Place H. Dunant, BP 38, 63001 Clermont-Ferrand France

[2] F. Hoffmann-La Roche Ltd. Pharmaceutical Division, Galenical & Analytical Development. CH-4070, Basel, Switzerland

E-Mails: emmanuel.scheubel@roche.com; marc.lindenberg@roche.com; eric.beyssac@u-clermont1.fr ; j-michel.cardot@u-clermont1.fr

*Author to whom correspondence should be addressed: Tel: +41616886357 ; Fax +41616870280

Abstract:

Standard compendia dissolution apparatus are the first choice for development of new dissolution methods. Nevertheless, limitations coming from the amount of material available, analytical sensitivity, lack of discrimination or biorelevance may warrant the use of non compendial methods. With this regard, the use of small volume dissolution methods offers strong advantages. The present study aims primarily to evaluate the dissolution performance of various drug products having different release mechanisms, using commercially available small volume USP2 dissolution equipment.

The present series of tests indicate that the small volume dissolution is a useful tool for the characterization of immediate release drug product. Depending on the release mechanism, different speed factors are proposed to mimic common one liter vessel performance. In addition, by increasing the discriminating power of the dissolution method, it potentially improves know how about formulations and on typical events which are evaluated during pharmaceutical development such as ageing or scale up. In this regard small volume dissolution is a method of choice in case of screening for critical quality attribute of rapidly dissolving tablets, where it is often difficult to detect difference using standard working conditions.

Key words: Dissolution, Small volume, Discrimination, Screening, Quality By Design

1. Introduction

Dissolution testing is a core performance test in pharmaceutical development and quality control. Dissolution testing more and more evolved to establish relationships with in vivo performance or with manufacturing Critical Quality Attributes (CQA) in the scope of Quality by Design (QbD)[12]. The overall goal is to better control product performance within the life cycle of a product. For this purpose the use of the classical USP dissolution working conditions using one liter vessel with basket (respectively USP1) and paddle (respectively USP2) are well established[13,14] and are used as first choice for development of a new dissolution method.

Nevertheless, limitations coming from the amount of material available, analytical sensitivity, lack of discrimination or biorelevance may warrant the use of non compendial methods. In particular in early phase development, during screening of drug candidates, formulation is often developed for studies in animals and dissolution should be ideally conducted using media simulating the gastrointestinal environment as well as in volumes in line with the animal physiology[15]. Another case in which a classical method is not well suited is for low dose drugs or if the analytical method is not sensitive enough to detect the amount of dissolved drug precisely due to low concentration of the drug in the formulation[16]. To overcome those problems the concept of small-volume dissolution arose recently due to the possibility of using smaller sample sizes and smaller volumes of media, offering various advantages in view of substance and material consumption[17] and can serve as a valuable tool for dosage form screening[18] or formulation selection in animals.

The present study aims primarily to evaluate the potential of commercially available small volume USP2 dissolution equipment for the dissolution of solid drug product. This miniaturized vessel/paddle equipment can be easily fitted, without hardware change or adaptation, on a classical USP2 system. For this purpose different kind of dissolution release mechanisms for solid drug products; immediate release (IR), extended release (ER) as well as low dose tablets, were screened using both standard (one liter) and small volume dissolution setup. Working conditions to achieve the same dissolution performance for both tests were sought using the small volume equipment. Attempts to generalize these dissolution working conditions for new products are discussed. The discriminating power of the method is stressed through one example of IR tablets by comparing the contribution of the small vessel dissolution on typical events faced during development such as aging and scale up versus compendial apparatus.

2. Experimental Section

2.1. Materials

Phosphate buffer, sodium chloride, 37% hydrochloric acid (fuming), 85% ortho-phosphoric acid, ethanol (99.9%) as well as HPLC grade methanol were purchased from Merck (Darmstadt, Germany). Water was obtained from a Milli-Q (Millipore, Milford, MA, USA) water purification system. For all tests GR grade material was used.

2.2. Methods

Dissolution experiments were performed using a Sotax AT7 smart apparatus (Sotax, Allschwill, CH). The small volume vessel is based on the USP one liter vessel setup, scaled down to be used with 50 mL to 200 mL of dissolution medium with an internal diameter of 40 mm. The Sotax small volume vessel is a single device and offers the advantage to be installed directly on existing equipment. A small paddle of 29 mm length fitted at 10 mm from bottom of the vessel is used. An overview of the small volume set up is presented Figure 1 and the different sizes of the small volume equipments are listed in Table 1. The investigations were conducted in 150 mL, working conditions that allow providing sink condition for all tested products.

The aim of the series of tests was to establish a relationship between the reference one liter vessel method (using 900 ml or 500 ml of media) and the small vessel. For this purpose the rotation speed of the small vessel system was varied from 50 rpm up to 150 rpm to evaluate the speed factor (sf) between both methods. All the tests were performed in triplicates for screening purpose and with 6 units during the evaluation of scale up and ageing with one example in order to confirm the early findings and assess the potential of the method during development. An overview of the dissolution working conditions for the classical one liter dissolution method is presented Table 2. The samples were collected semi automatically, filtrated and measured according to USP or by validated UV or HPLC methods. For all tests the same dissolution apparatus was used.

2.3. Model compounds

Five different products exhibiting different type of release rates were chosen. Both Performance Verification Test tablets (prednisone and salicylic acid, disintegrating and non disintegrating tablets respectively) were bought at USP, Rockville USA. Experimental IR formulations and ER tablet formulations were supplied by Roche Pharmaceutical Research

department, Basel, CH. The ER tablets formulations were produced by wet granulation using different amounts of HPMC to achieve, 4 hours (ER4H) and 8 hours (ER8H) release profiles. The IR formulations are either immediate release, low dose tablet (IR(1)) or a very rapidly dissolving tablet IR(2), both exhibiting 85% dissolved within 15 minutes in classical conditions.

The API of these five drug products exhibit high or low solubility according to the biopharmaceutical classification system (BCS)[19]. However the medium chosen during these investigations were set up in order to reach sink conditions in 150 ml. For each product, the same medium was used for the one liter and for the small vessel testing. An overview of the tablet types and properties is listed in Table 2.

For IR(2), comparison after storage 3 months at 25°C/ 60 % relative humidity (r.h.) and 40°C/75% r.h. according to ICH conditions and after scale up (8kg to 15kg) were performed using both methods.

2.4. In vitro dissolution test comparison

For the screening purpose of the study, in addition to a visual comparison of the dissolution profiles, where the shape and the plateau of the curves were estimated, the closeness of the profiles was assessed by calculating the ratio of percent dissolved at each time point according to equation 1 and the mean ratio for all sampling points was assessed using equation 2.

$$\Theta(t) = D_{small}(t) / D_{ref}(t) \qquad \text{eq1}$$

$$\Theta_{mean} = \frac{\sum_{t=1}^{n} R(t)}{n} \qquad \text{eq2}$$

$\Theta(t)$ represents the ratio at time t, D_{small} the percent dissolved for the small volume method and Dref the percent dissolved for the reference method (so called one liter). Θ_{mean} represents of mean of the $\Theta(t)$.

A Θ_{mean} close to one are sought with a ratio stable all along the profile. Θ_{mean} above 1 would mean that the profiles have the tendency to be faster than the reference. Θ_{mean} below 1 would mean that the profiles have the tendency to be slower than the reference. Applying such a ratio assumes that the dissolution curves exhibit similar profiles with only a difference in the rate of dissolution. The f2 factors[20] were calculated on the mean dissolution values as an additional factor to the Θ_{mean}.

3. Results and Discussion

The Figures 2 to 8 show the mean dissolutions profiles of all tested variants and Table 3 shows the mean of the ratios. Similar findings were found for the ratios and the f2 factors. No coning or mounting was observed using the small volume vessel except for the prednisone disintegrating tablets what was also seen for the one liter vessel. Similar curves shapes were observed for prednisone, salicylic acid as well as for ER tablets. Slight different curves shape and time to reach the plateau were observed for the IR(1) and IR(2) tablets. For all dissolution experiments, the observed standard deviations (SD) are low (maximum of 6% at first sampling point and below 5% for the next sampling points). The SD are similar for both small volume and one liter methods through the entire profiles.

The small volume vessels using the identical rotation speed as for the one liter vessel showed a lower percent of drug dissolved for most of the methods except for the slowest ER8H using paddle at 50 rpm.

For prednisone (Figure 2), a small vessel/paddle at 125 rpm results in a similar profile compared to the USP paddle 50 rpm method. This corresponds to a speed factor (sf) of 2.5 (sf = 2.5).

For salicylic acid non-disintegrating tablets (Figure 3), a small vessel/paddle at 150 rpm results in a similar profile to the USP paddle 100 rpm method (sf =1.5).

For the extended release tablets ER4H and ER8H (Figure 4), the impact of the small vessel/paddle setup is less pronounced. By varying the rotation speed from 50 to 100 rpm, similar profiles can be observed and the ratios remain very close.

For the IR(1) tablets (Figure 5), both motion speeds at 100 rpm and 125 rpm using small vessel/paddle result in a similar profile to the one liter method with paddle at 50 rpm (sf = 2.5).

For the IR(2) tablets (Figure 6), small vessel/paddle at 125 rpm results in similar profiles to the one liter method at paddle 50 rpm method (sf =2.5).

The comparison of samples after storage (Figure 7) does not show difference whereas after scale up (Figure 8) a new trend is visible only using the small vessel at 50 rpm.

All those results are summarized in Table 4.

These investigations clearly showed that using the small vessel set up, equivalent or higher rotational speeds are necessary to obtain similar dissolution rates when compared to the one liter vessel. Speed factors from 1 to 2.5 have been observed (see Table 4).

A theoretical calculation of the rotation speed needed for the small paddle to reach the velocity of the large paddle at 50 rpm was performed based on the differences of the paddle sizes (Table

5)[21]. A corresponding rotation speed of 121 to 129 rpm was found. This difference corresponds to a speed factor of 2.5.

A speed factor of 1.5 was observed for salicylic acid tablets and 1 to 2 for the ER formulations. A speed factor of 2.5 was observed for the IR formulations (prednisone , IR(1) and IR(2)) indicating that the working conditions to obtain the performance of one liter vessels in small vessels clearly depend on the type of release mechanism.

In case of fast dissolving IR formulation as presented in this paper, one of the main factors to take into account beside the intrinsic properties of the API (e.g. solubility) is the rate of renewal of the dissolution media in contact with the API. Based on Noyes Whitney equation[22] and diffusion layer term[23,24], it is directly in relation with the rotation speed of the dissolution method.

In case of the salicylic acid tablets or the ER formulations the limiting factor is not driven only by dissolution properties of the API but rather by the design of the formulation (e.g. erosion/diffusion[25] and, therefore the characteristics of the formulation are less dependent to the renewal of the media as soon as this renewal is faster than the release rate[26,27,28]. This phenomenon is emphasized in vitro for the longer releasing tablets. In our example for the ER8H no difference could be observed between both methods and that independently of the rotation speed in small vessels. Diffusion controlled tablets would then not be impacted by the hydrodynamics[29] and the speed factor may come close to 1.

For tablets impacted by small volumes, a higher discriminating power may be expected by measuring of rapidly dissolving tablets using small vessel at 50 rpm or less. In this case 50 rpm in small vessel would correspond approximately to 20 rpm (50 rpm divided by sf 2.5) in one liter vessel which would be out of the range of standard performance verification test of the apparatus.

Based on this observation, further investigation were tried with the IR(2) tablets. At 50 rpm with the small vessel/paddle, the differences after manufacturing scale up is more pronounced than with the one liter vessel (Figure 7), whereas no significant change can be observed after storage under different temperatures (Figure 8). These differences are highlighting a possible change of the intrinsic quality of the tablets after manufacturing scale up, whereas the product seems to be very stable after 3 months storage even under stress storage conditions and using the most discriminating dissolution method.

The significance of the observed difference does not mean that a change in *in vivo* performance should be expected, the profiles remain very rapidly dissolving and both tablets should be completely dissolved before gastric emptying[30]. However this difference points out a change in the tablets properties after scale up and further investigations on manufacturing

parameters and resulting solid state properties may be initiated. In this regard the small vessel dissolution method supports a better process understanding and is in line with a QbD approach. Results from the present series of tests indicated that the small paddle apparatus might be a useful tool in characterizing drug release profiles under standard test conditions mainly to IR and disintegrating tablets as it was shown to be more discriminant.

Takano et al[31] showed that small volumes can also be applied for low soluble molecules even under non sink conditions

During development of small volume method, it is important to take into account that the current small or low volume vessels are non compendial. The commercially available vessels are well defined[32] but there are still differences from supplier to supplier. It was demonstrated that differences in the actual compendia apparatuses existed between suppliers even if within the standardized dimensions and that those differences affected marginally the results[33]. In case of small volume vessels there is no currently fixed dimension between suppliers. This means that each investigation should be carry out specifically and that transfer is more complicated than using classical pharmacopeia one litre vessel.

The discriminating power of the small volume method seems more pronounced for IR compared to ER formulations. It is therefore recommended to systematically integrate small volume methods in the screening of new methods for IR formulation.

4. Conclusion

This limited set of data clearly showed that the small volume apparatus is a useful tool in the characterization of solid drug product dissolution profiles. It can be easily installed in a standard laboratory, it uses standardised working conditions and can be set up to fit to the common one litre vessel performance when the dissolution method is not rugged enough for instance with an analytical method having an improper sensitivity. In addition beside the advantage of using smaller volumes of media, it potentially allows to expand the discriminating power of a method by applying gentle agitation which is particularly important for IR and disintegration tablets. Only two IR tablets within sink conditions were exemplified and further tests should be initiated to consolidate these first outcomes. Nevertheless these data taken as starting point showed that this approach improves know how about formulations, the process and is a method of choice in case for instance of screening for CQA of rapidly dissolving tablets where it is often difficult to detect difference using standard working conditions.

Conflict of Interest

There is no conflict of Interest.

Acknowledgement

The authors acknowledge: C. Keiflin, P. Wininger, C. Maureta, M. Brach, Y. Ducommun for technical assistance and Dr. B. Fischer for managerial support.

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Figure 14 : Small volume vessel setup with small Paddle. On left side, the compendial one liter vessel with paddle.

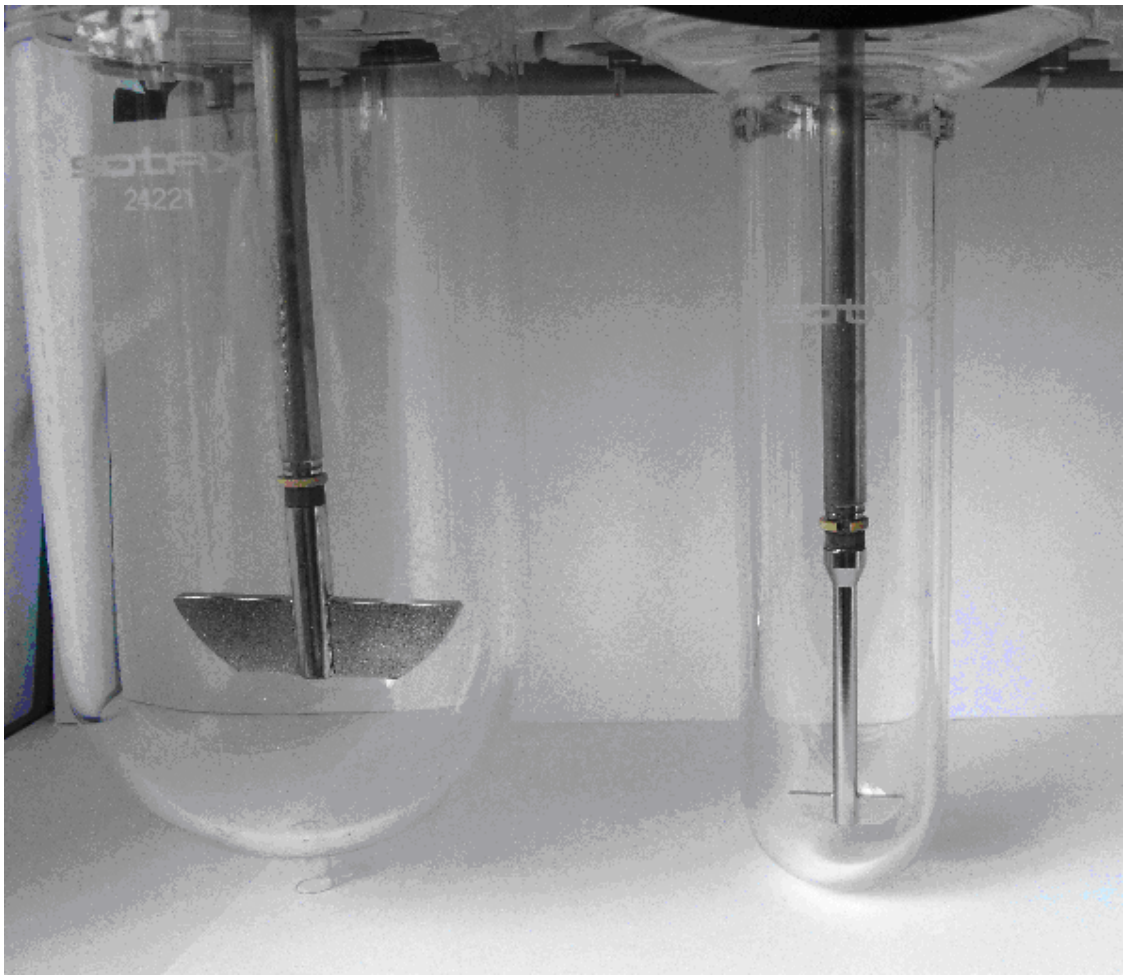


Figure 15: Prednisone tablets with small vessel / paddle versus USP method with one liter vessel.

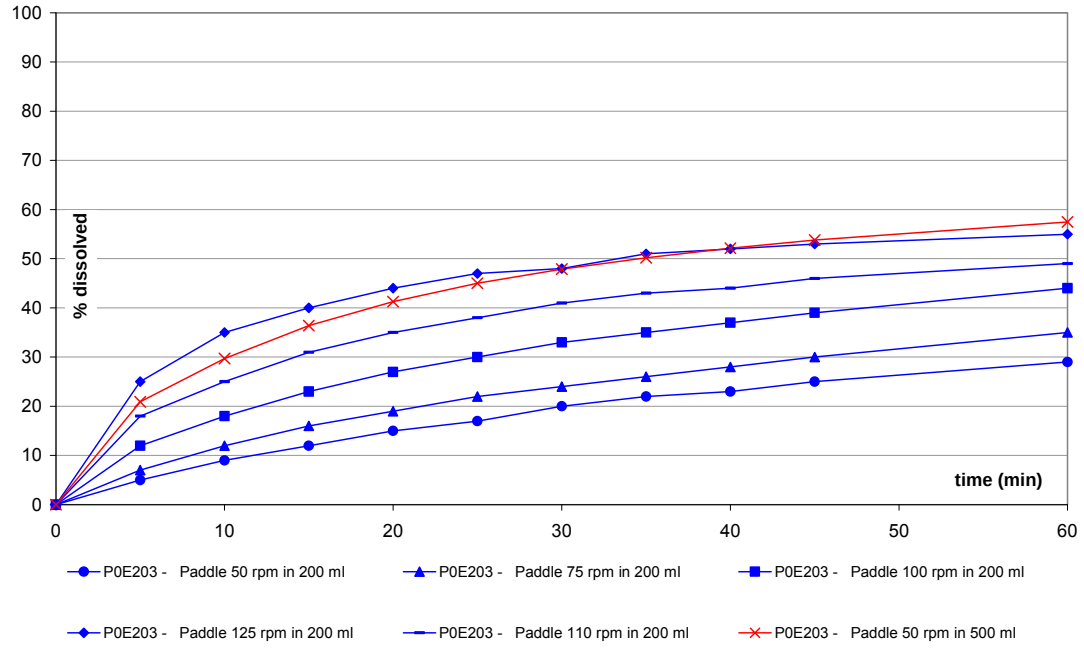


Figure 16 : Salicylic acid tablets with small vessel / paddle versus USP method with one liter vessel.

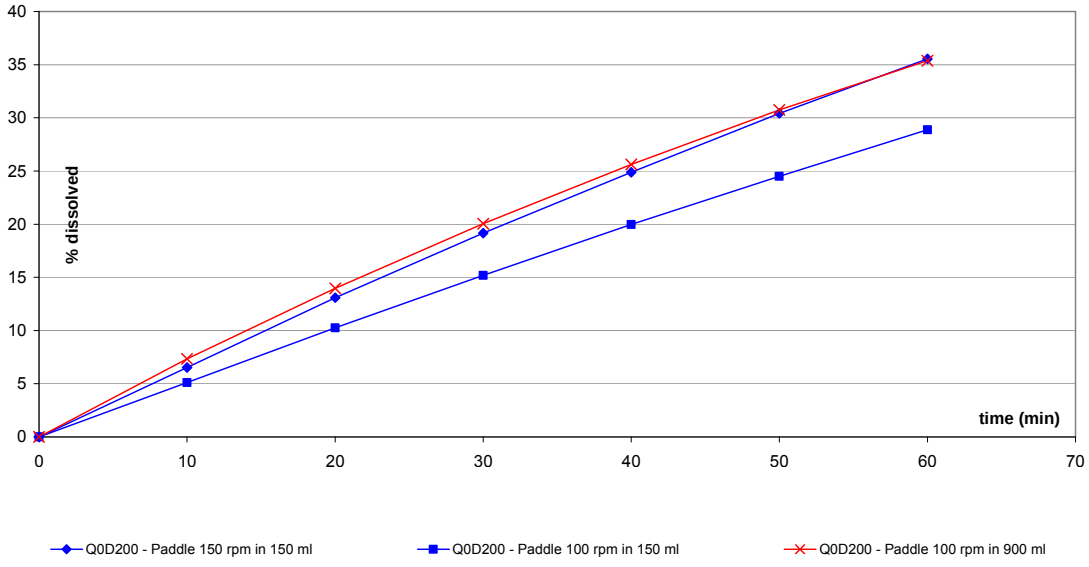


Figure 17 : ER4H and ER8H tablets: comparison of small vessel/paddle versus one liter Vessel

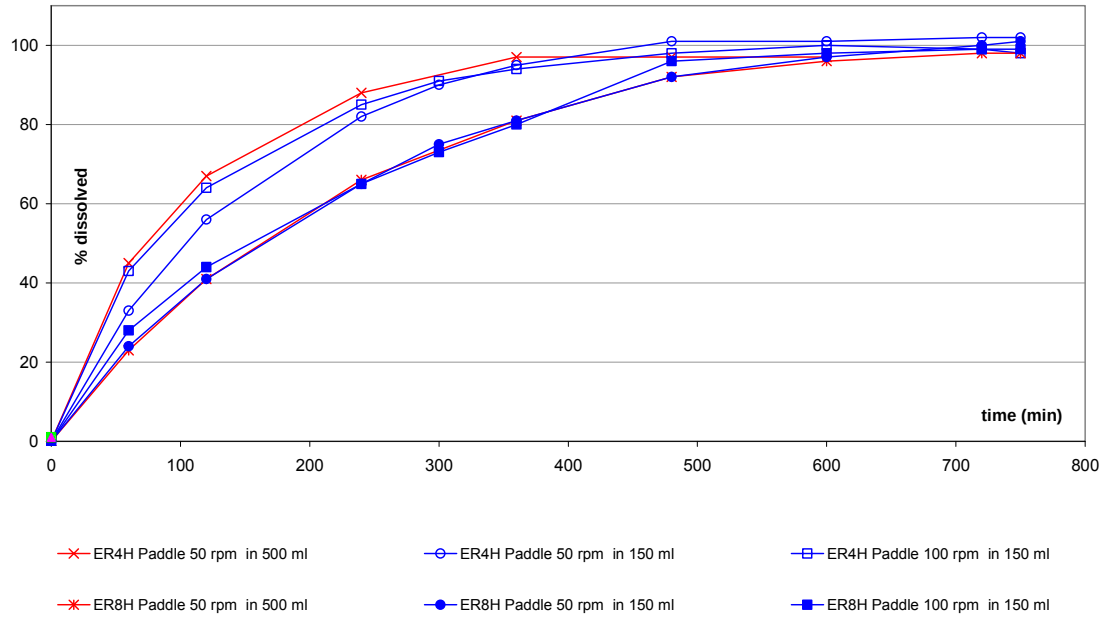


Figure 18 : IR(1) tablets: comparison of small vessel/paddle versus one liter Vessel

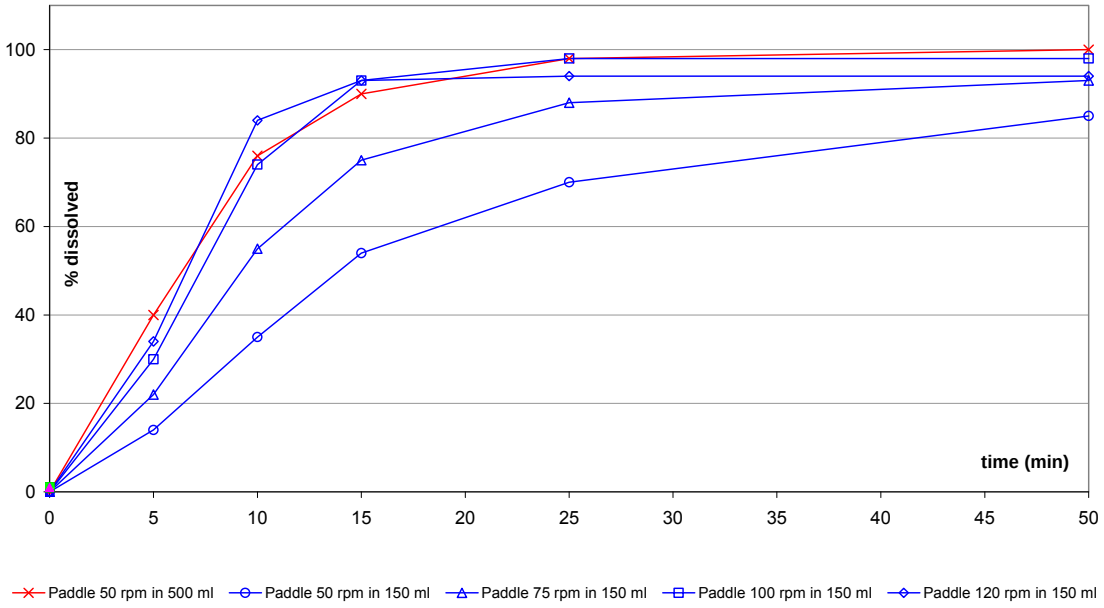


Figure 19 : IR(2) tablets: comparison of small vessel/paddle versus one liter Vessel

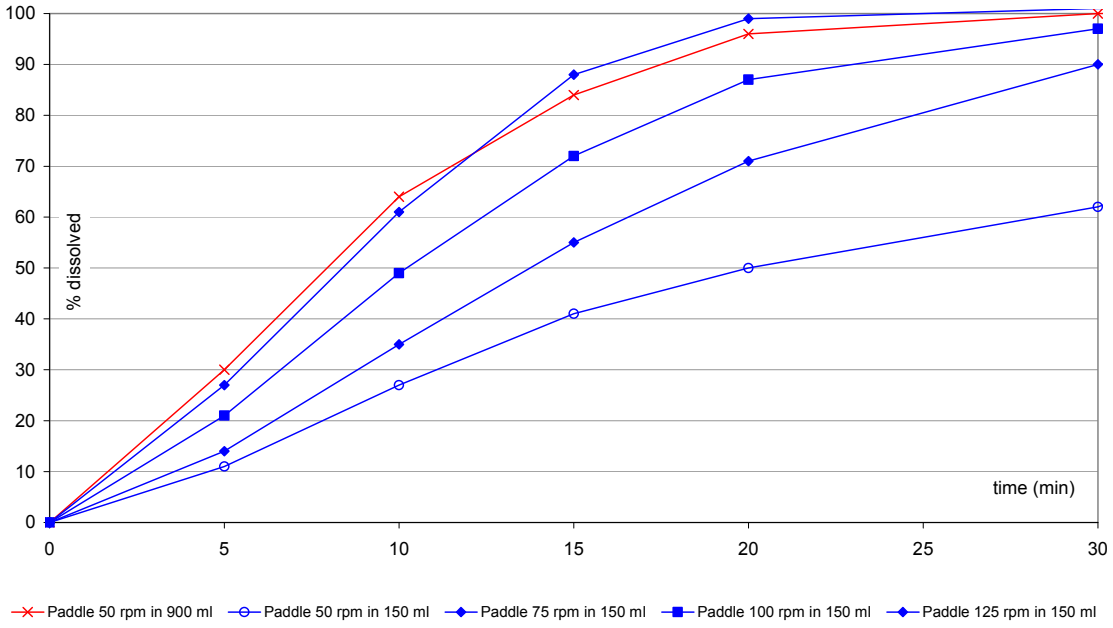


Figure 20 : IR(2) tablets: comparison after scale up using small vessel/paddle

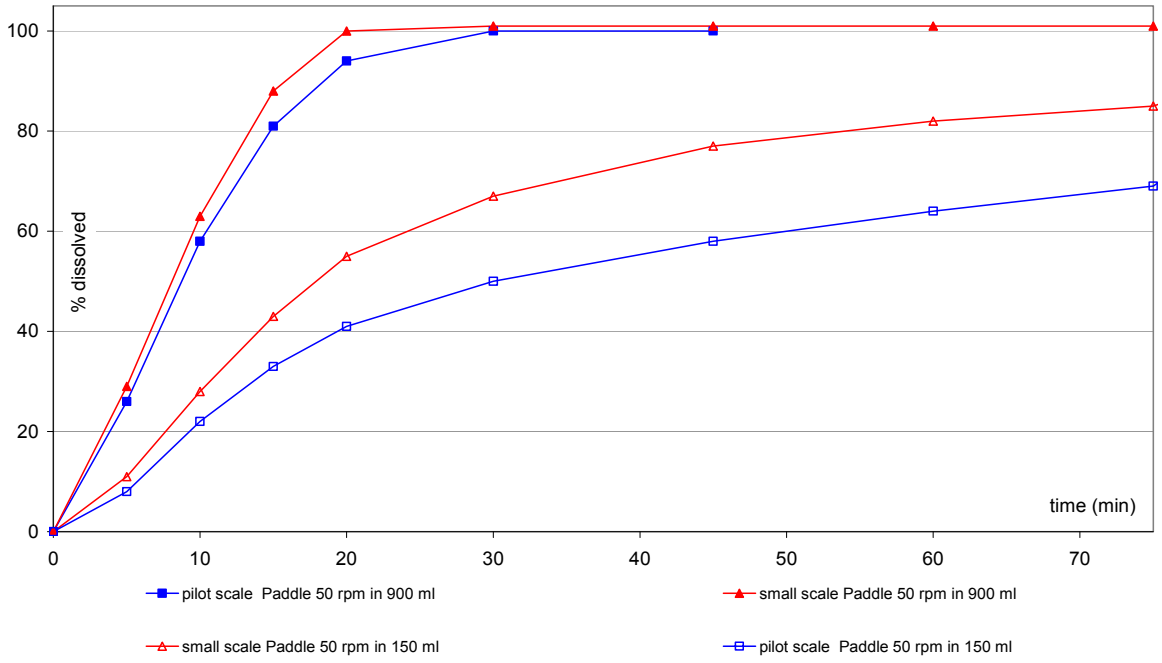
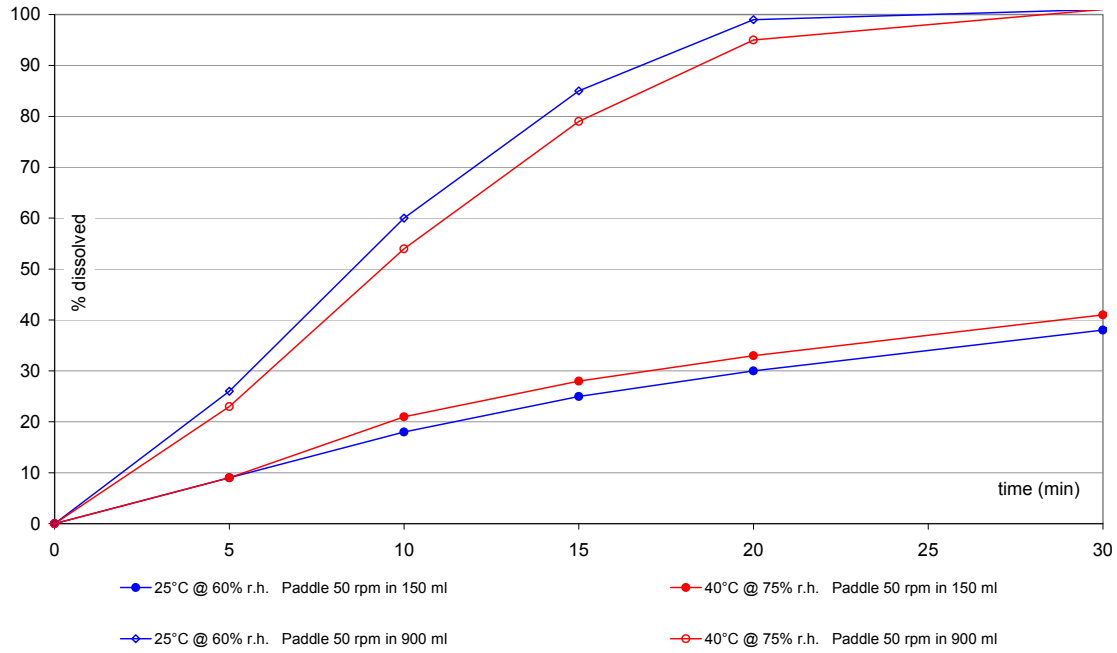


Figure 21 : IR(2) tablets: comparison after storage using small vessel/paddle



List of Tables

Table 5: Dissolution – Difference in Dimension (mm) of the small and USP Vessels and Paddle.

	USP one liter vessel	**Small volume Apparatus**
Vessel		
Height	168 ± 8	185
Internal diameter	102 ± 4	4
Paddle		
Blade Upper chord	74.0 ± 0.5	29
Blade Lower chord	42.0 ± 1.0	18
Height	19.0 ± 1.0	7.5
Distance from the bottom	25 ± 2	10

Table 6: Overview of the tablets and release mechanisms tested using both dissolution methods

Product	**Strength (mg)**	**BCS class**	**Dissolution method with one liter vessel**		**Release mechanism**	**Tablets types**
Prednisone	10 mg	1	500 mL	Paddle 50 rpm	IR	Disintegrating
Salicylic acid	300 mg	3	900 mL	Paddle 100 rpm	ER	Non-disintegrating
ER4H / ER8H	1 mg	2	500 mL	Paddle 50 rpm	ER	Erosion-Diffusion
IR(1)	0.075 mg	1	500 mL	Paddle 50 rpm	IR	Disintegrating
IR(2)	50 mg	2	900 mL	Paddle 50 rpm	IR	Disintegrating

ER = Extended Release; IR = Immediate Release

Table 3: Mean of ratio (Θ_{mean}) percent dissolved between small and one liter dissolution at different rotation speeds. Best values are in bold

Product	**Reference Method**	**Small vessel rotation speed**					
		50 rpm	**75 rpm**	**100 rpm**	**110 rpm**	**125 pm**	**150 rpm**
Prednisone	Paddle 50 rpm	0.39	0.48	0.67	0.85*	**1.05***	-
Salicylic acid	Paddle 100 rpm	-	-	0.76*	-	-	**0.96***
ER4H	Paddle 50 rpm	0.93*	-	**0.98***	-	-	-
ER8H	Paddle 50 rpm	1.01*	-	**1.05***	-	-	-
IR(1)	Paddle 50 rpm	0.59	0.79	0.95*	-	**0.98***	-
IR(2)	Paddle 50 rpm	0.57	0.71	0.86	-	**0.99***	-

** indicates the f2 factors between small and one liter vessel with a value above 50.*

Table 4: Found rotation speed factors using small vessel versus one liter vessel to reach the same performance.

Tablet type	Product	Dissolution method	Rotation speed using one liter vessel	Rotation speed using small vessel	*Rotation speed Factor (sf)*
disintegrating	Prednisone	Paddle	50	125	2.5
disintegrating	IR(1)	Paddle	50	125	2.5
disintegrating	IR(2)	Paddle	50	125	2.5
Non-disintegrating	Salicylic acid	Paddle	100	150	1.5
Non disintegrating	ER4H	Paddle	50	50-100	1-2
Non disintegrating	ER8H	Paddle	50	50-100	1-2

Table 5: Theoretical calculation of hydrodynamics difference between small paddle and large paddle.

		Equation	Length on top of the paddle		Length on bottom of the paddle		
			small	**large**	**small**	**large**	units
Rotation	R		100.00	50.00	100.00	50.00	rpm
Frequency	F	R/60	1.67	0.83	1.67	0.83	Hz
Periodicity	T	1/F	0.60	1.20	0.60	1.20	s
Angular velocity	W	2pi/T	10.51	5.25	10.51	5.25	rad.s-1
1/2 lenght	R		14.50	37.25	8.70	21.00	mm
Linear speed on top of the paddle	**V**	**R*W**	**152.33**	**195.66**	**91.40**	**110.31**	**cm s-1**
Calculation of the angular velocity for the small paddle	W		13.49		12.68		rad.s-1
Periodicity	T		0.47		0.50		s
Frequency	F		2.15		2.02		Hz
			128.86	=>**129**	121.07	=>**121**	rpm

Small Volume Dissolution Testing as Powerful Method during Pharmaceutical Development

Supplement 1:" Small Volume Dissolution Testing using Basket method".

Introduction

In connection with the investigations performed with small volume associated with small paddle (resp. USP2) as described in paper 3, additional testing's were initiated for the basket method (resp. USP1). A difference between paddle and basket was observed in the literature using classical one liter vessel where the mixing ability of the paddle is higher than basket at the same operating speed (Morihara et al., 2002; D'Arcy et al., 2005;D'Arcy et al., 2006).The goal of the study was as sought for the paddle, to explore if a relationship small – large volume can be found.

Materiel and methods

The tablets defined for the PVT were investigated using the basket method as reference. Dissolution experiments were performed using a Sotax AT7 *smart* apparatus (Sotax, Allschwill, CH). The small volume vessels described in paper 3 were further utilised. Standard USP baskets fitted at 10 mm from bottom of the vessel have been used. An overview of the small volume set up for the basket (and paddle for comparison) is presented Figure 1 and the different sizes of the small volume equipments are listed in Table 1 of paper 3 (data not repeated). The tests were conducted in 150 mL that allowed providing sink condition for all tested products. In order to match the reference profile performed in a one liter vessel, working conditions were screened by varying the rotation speed from 50 rpm up to 150 rpm depending on the found profiles. An overview of the dissolution working conditions for the classical one liter dissolution set up is presented Table 1. All the tests were performed in triplicates. The samples were collected semi automatically, filtrated and measured according to USP or by validated UV or HPLC methods. For all the tests the same dissolution apparatus was used.

The similarity of the profiles were assessed using the ratio mentioned in paper 3 and the f2 similarity factor (as well with the limitations mentioned in paper 3)

Results

The Figures 2 and 3 show the mean dissolutions profiles of both variants and Table 2 shows the mean of the ratios calculated for each drug product resp. the f2 factors, using the one liter method as reference.

Similar curves shapes for small volume and one liter vessel were observed for prednisone and salicylic acid respectively.

For all dissolution experiments, the observed standard deviations (SD) are low (maximum of 5% at first sampling point and below for the next sampling points). The SD are similar for both small volume and one liter methods through the entire profiles. The triplicate determination allows therefore performing a reasonable profile comparison using the mean values with enough confidence for a screening.

For prednisone (Figure 2) a similar profile to the basket 50 rpm USP method can be achieved using a rotation speed of 75 rpm using the small volume vessel (speed factor 1.5). The data are summarized Table 1 and Table2.

For salicylic acid non-disintegrating tablets (Figure 3), with the USP basket method at 100 rpm, comparable profiles can be observed for 100 rpm and 150 rpm using the small vessels (speed factor 1 or 1.5). The results are summarized in the Table 1.

Table 1: Mean of ratio percent dissolved between small and one liter dissolution at different rotation speeds. Best values are in bold

Product	Reference Method	Small vessel rotation speed			
		50 rpm	75 rpm	100 rpm	150 rpm
Prednisone	basket 50 rpm	0.82	**1.09***	-	-
Salicylic acid	basket 100 rpm	-	-	**0.94***	1.10*

** indicated the f2 factors between small and one liter vessel where a value above 50 can be found.*

Table 2: Found rotation speed factors using small vessel versus one liter vessel to reach the similar performance (best matched).

Tablet type	Product	Dissolution method	Rotation speed using one liter vessel	Rotation speed using small vessel	*Rotation speed Factor*
desintegrating	Prednisone	basket	50	75	1.5
Non desintegrating	Salicylic acid	basket	100	100	1

Figure 1 : Small volume vessel setup with small Paddle and Basket. On left side, the compendial one liter vessel with paddle.

Figure2: Prednisone tablets with small volume vessels and basket versus USP method with one liter vessel.

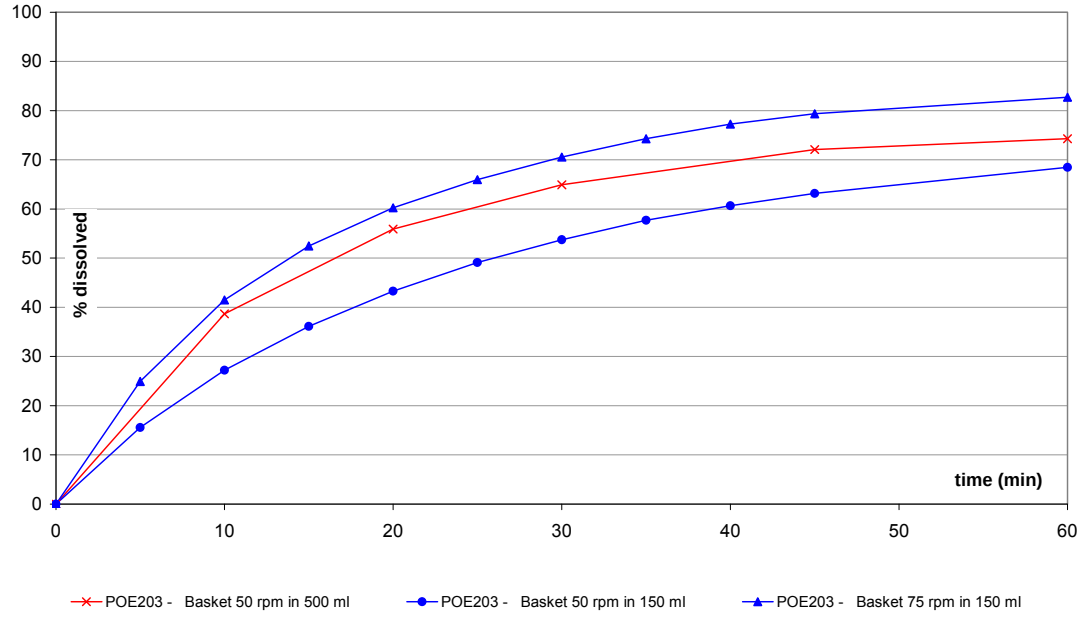
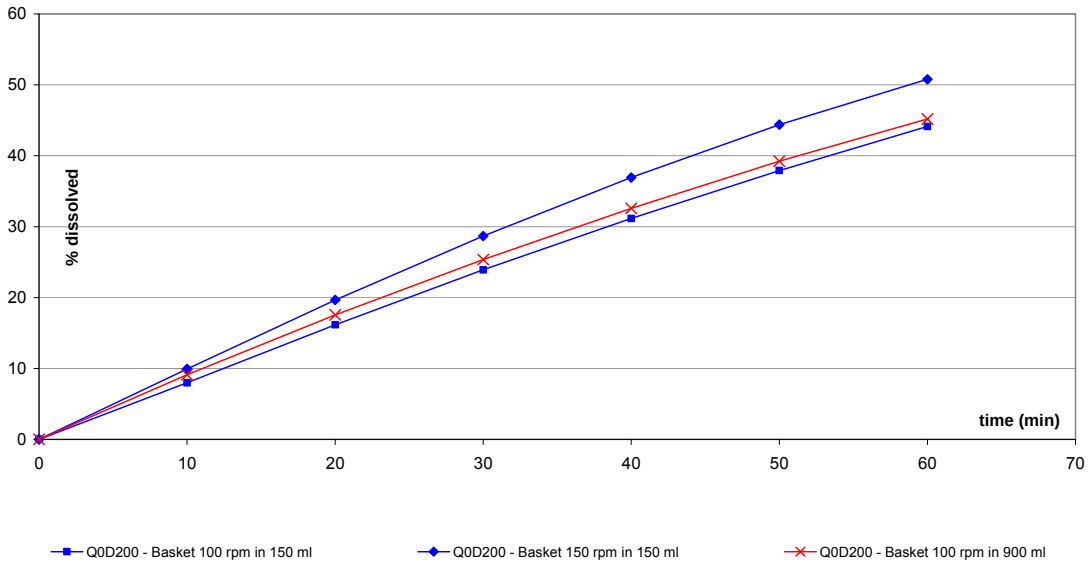


Figure 3 : Salicylic acid tablets with small volume vessel and basket versus USP method with one liter vessel.



Discussion

These investigations clearly showed that using the small vessel set up, equivalent or higher rotational speeds are necessary to obtain similar dissolution rates when compared to the one liter vessel.

For test performance tablets (prednisone) a common speed factor of 1.5 could be estimated between the two vessels using basket. For test performance tablets (salicylic acid) no clear difference can be observed. As for the paddle investigations, the observed response clearly depends on the tablets type. For the prednisone however, the difference observed is primary due to

Conclusion

The use the small volume vessel associated with basket can be easily installed in all standard laboratories. However it shows less advantage in view of discriminating power in comparison to the mini paddle. This approach using basket needs definitively further investigations with broader type of products. As well new testing on small basket recently available should be initiated to fine tune this approach.

Acknowledgement

The authors acknowledge: C. Keiflin, P. Wininger, C. Maureta, M. Brach, Y. Ducommun for technical assistance and Dr. B. Fischer for managerial support.

Reference supplement 1

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Tenoxicam–Methylparaben Cocrystal Formation in Aqueous Suspension Formulation

Nicole Wyttenbach[1], André Alker[2], Olaf Grassmann[2], Emmanuel Scheubel[3]

[1]Pharmaceutical & Analytical R&D, [2]Discovery Technologies, [3]Galenical and Analytical Development, F. Hoffmann-La Roche Ltd., Basel, Switzerland

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Introduction

Aqueous suspension formulations are often used for oral drug administration in nonclinical pharmacokinetic, pharmacodynamic, or toxicology studies or in phase 1 clinical trials, and are frequently used as pediatric or veterinary dosage forms. The investigation of an experimental suspension formulation with tenoxicam (TXM) revealed the unexpected presence of a new solid form. Further characterization of this solid form indicated that TXM formed a cocrystal with methyl-4-hydroxybenzoate (methylparaben), a preservative in the suspension vehicle.

TXM (Fig. 1) is a nonsteroidal anti-inflammatory drug (NSAID) which is used to relieve inflammation, swelling, stiffness, and pain associated e.g. with rheumatoid arthritis or osteoarthritis. Four polymorphs of TXM are known. Form III is the thermodynamically most stable form at ambient conditions. A number of salts are known and the formation of different solvates has been reported [1].

Purpose

➔ Characterization of a new TXM cocrystal with methylparaben formed in suspension formulation.

Materials and Methods

Materials

Tenoxicam (form III) was purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland) and was micronized by air jet milling. Methylparaben was purchased from Fluka (Buchs, Switzerland). All other chemicals used were of standard research grade.

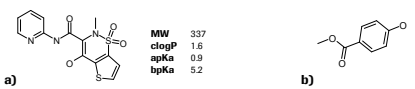


Figure 1. Molecular structures of tenoxicam (a) and methylparaben (b).

Preparation of TXM Suspension

The suspension formulation was prepared by suspending 4 mg/g TXM in an aqueous vehicle containing 0.5% HPMC (Methocel K4M Premium USP/EP, Colorcon Limited), 0.2% Tween 80, 0.18% methylparaben, 0.02% propylparaben.

Preparation of TXM Cocrystals with Methylparaben

TXM cocrystals with methylparaben were obtained by slow evaporation of a solution containing 50 mg TXM and 23 mg methylparaben dissolved in 10 mL chloroform. The amounts of drug and cocrystal former used corresponded to a molar ratio of 1:1. Larger crystals were isolated and were used for single crystal x-ray characterization.

Cocrystal Characterization

Polarization Microscopy

A polarization microscope (Zeiss Axiolab) was used and the suspension samples were investigated without further sample preparation.

Thermal Analysis

Differential scanning calorimetry (DSC) was performed with a Mettler-Toledo differential scanning calorimeter DSC 1, thermo gravimetric analysis (TGA) was performed on a Mettler-Toledo TGA/DSC 1 STARe system (Mettler-Toledo AG, Greifensee, Switzerland). The measurements were performed at a heating rate of 10°C/min using nitrogen as a protective gas.

X-ray powder diffraction (XRPD)

XRPD patterns were recorded at ambient conditions with a STOE STADI P diffractometer (CuKα1 radiation, primary Ge-monochromator, position sensitive detector (PSD), 3° to 42° 2-theta angular range, 0.5° 2-theta PSD step width, 40 s per step measurement time). The samples were analyzed without further processing (e.g. grinding or sieving) of the material.

Single crystal X-ray diffraction

The crystal structure was obtained from synchrotron data (Swiss Light Source (SLS) synchrotron, PX II beamline). The structure was solved and refined with standard crystallographic software (ShelXTL from Bruker AXS,Karlsruhe).

In vitro Dissolution

The dissolution tests were conducted with a miniaturized USP-2 method (Sotax AT7 smart dissolution station) in 200 mL glass vessels. The dissolution experiments were carried out under non-sink conditions in triplicate with 1.5 mL TXM suspension (~6 mg TXM) in 150 ml simulated gastric fluid (SGF) pH 2 [2] at 25°C with freshly prepared TXM suspension and with suspension stored at 4°C for 1 week. The paddle speed was 25 rpm and the drug concentration was directly determined by UV online detection at 377 nm.

Ultra Performance Liquid Chromatography (UPLC)

Analysis was performed on a Waters Acquity™ system, equipped with an Acquity UPLC™ BEH C18 column (2.1x50 mm, 1.7 mm particles). A linear gradient with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) was run with 20% B to 100% B during 0.5 min, flow rate 0.75 ml/min, total run time 1.2 min, column temperature 30°C, UV detection at 377 nm.

Results

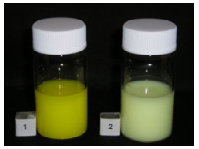
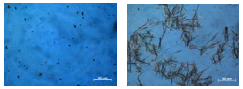


Figure 2. TXM suspension freshly prepared (1) and after storage for 1 week at 4°C (2).



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Oral suspension formulations with TXM exhibited a visual change in color from yellow (freshly prepared suspension) to light yellow (after storage) (Fig. 2). Microscopic analysis revealed the formation of crystal needles (Fig. 3). Analysis of the isolated needles by XRPD and UPLC measurements indicated the formation of TXM cocrystals with methylparaben, a commonly used preservative in oral suspension formulations.

The novel TXM methylparaben (1:1) cocrystal phase was confirmed by its unique thermal and XRPD properties and by single crystal X-ray diffraction (Figs. 4-6). The in vitro dissolution of the suspension with TXM-methylparaben cocrystals (TXM suspension after storage) was significantly improved compared to the freshly prepared suspension with TXM (Fig 7).

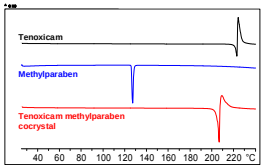

Figure 4. DSC curves of TXM (top), methylparaben (middle), and TXM methylparaben cocrystal (bottom).

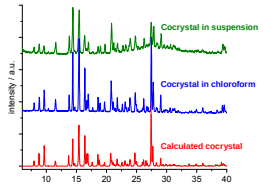

Figure 5. Overlay of XRPD patterns of TXM methylparaben cocrystals formed in suspension (top) and prepared in chloroform (middle), and XRD cocrystal pattern calculated from single crystal structure (bottom).

Table 1. Crystallographic data of the TXM cocrystal with methylparaben (1:1).

	TXM cocrystal with methylparaben (1:1)
Empirical formula	C21 H19 N3 O7 S2
Formula weight	489.51
Temperature [K]	89
Wavelength [Å]	0.80
Crystal system	Triclinic
Space group	P-1
a [Å]	9.0600 (18)
b [Å]	10.910 (2)
c [Å]	12.170 (2)
α [deg]	67.75 (3)
β [deg]	72.92 (3)
γ [deg]	74.65 (3)
Volume[Å^3]	1048.1 (4)
Z	2
Calculated density [g/cm^3]	1.551
Absorption coefficient [mm^{-1}]	0.306
F(000)	508
Crystal size	0.1 x 0.05 x 0.02 mm
Reflections collected	14432
No. of unique reflections	3719
R(int)	0.0571
Final R indices [I>2sigma(I)]	R1 = 0.0477, wR2 = 0.1327
R indices (all data)	R1 = 0.0512, wR2 = 0.1360
Goodness-of-fit on F^2	1.072
Data deposition	Roche CSD structure No. 1776

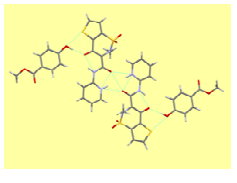

Figure 6. Crystal packing in TXM methylparaben cocrystal. Light blue dotted lines represent hydrogen bonds. The hydrogen bonding between drug and ligand consisted of an interaction between the hydroxy group of the methylparaben molecule and the pentacyclic sulfur as well as the deprotonated oxygen of TXM which is present in its zwitterionic form [3].

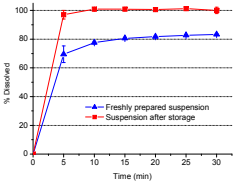

Figure 7. Dissolution profiles of freshly prepared TXM suspension and after conversion to TXM methylparaben cocrystals (i.e. after storage of the suspension for 1 week at 4°C).

Conclusions

➔ New cocrystal of TXM with methylparaben identified in oral suspension formulation

➔ Cocrystal formation confirmed by polarization microscopy, TGA, DSC, UPLC, XRPD, and single crystal X-ray diffraction

➔ Cocrystallization of TXM with methylparaben was associated with an improvement of the in vitro dissolution behaviour of TXM

➔ Cocrystallized preservatives possibly do no longer exhibit antimicrobial activity (preservatives must be dissolved in sufficient concentration to be effective)

➔ Cocrystallization with preservatives can have a strong impact on the performance and on the microbiological quality of the drug product

➔ Suspension formulations should regularly be monitored for solid form changes over a certain period of time in the development phase, e.g. by periodical XRPD or Raman measurements

➔ Commonly used preservatives should be included in cocrystal screening programs

References

[1] R.G. Cantera et al., Solid Phases of Tenoxicam. J. Pharm. Sci. 91, 2002, 2240-2251

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[3] M.R. Caira et al., Zwitterionic nature of tenoxicam: Crystal structures and thermal analyses of a polymorph of tenoxicam and a 1:1 tenoxicam:acetonitrile solvate. J. Pharm. Sci. 84, 1995, 884-888

The poster , presented at the AAPS annual meeting 2009 (W4326) , was enlarged in three parts for a better visibility

Introduction

Aqueous suspension formulations are often used for oral drug administration in nonclinical pharmacokinetic, pharmacodynamic, or toxicology studies or in phase 1 clinical trials, and are frequently used as pediatric or veterinary dosage forms. The investigation of an experimental suspension formulation with tenoxicam (TXM) revealed the unexpected presence of a new solid form. Further characterization of this solid form indicated that TXM formed a cocrystal with methyl-4-hydroxybenzoate (methylparaben), a preservative in the suspension vehicle.

TXM (Fig. 1) is a nonsteroidal anti-inflammatory drug (NSAID) which is used to relieve inflammation, swelling, stiffness, and pain associated e.g. with rheumatoid arthritis or osteoarthritis. Four polymorphs of TXM are known. Form III is the thermodynamically most stable form at ambient conditions. A number of salts are known and the formation of different solvates has been reported [1].

Purpose

➔ Characterization of a new TXM cocrystal with methylparaben formed in suspension formulation.

Materials and Methods

Materials

Tenoxicam (form III) was purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland) and was micronized by air jet milling. Methylparaben was purchased from Fluka (Buchs, Switzerland). All other chemicals used were of standard research grade.

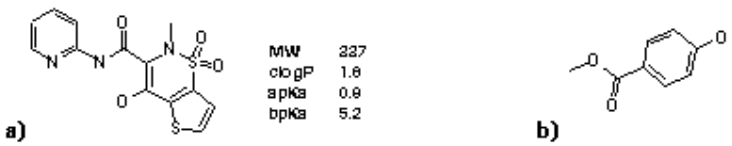


Figure 1. Molecular structures of tenoxicam (a) and methylparaben (b).

Preparation of TXM Suspension

The suspension formulation was prepared by suspending 4 mg/g TXM in an aqueous vehicle containing 0.5% HPMC (Methocel K4M Premium USP/EP, Colorcon Limited), 0.2% Tween 80, 0.18% methylparaben, 0.02% propylparaben.

Preparation of TXM Cocrystals with Methylparaben

TXM cocrystals with methylparaben were obtained by slow evaporation of a solution containing 50 mg TXM and 23 mg methylparaben dissolved in 10 mL chloroform. The amounts of drug and cocrystal former used corresponded to a molar ratio of 1:1. Larger crystals were isolated and were used for single crystal x-ray characterization.

Cocrystal Characterization

Polarization Microscopy

A polarization microscope (Zeiss Axiolab) was used and the suspension samples were investigated without further sample preparation.

Thermal Analysis

Differential scanning calorimetry (DSC) was performed with a Mettler-Toledo differential scanning calorimeter DSC 1, thermo gravimetric analysis (TGA) was performed on a Mettler-Toledo TGA/DSC 1 STARe system (Mettler-Toledo AG, Greifensee, Switzerland). The measurements were performed at a heating rate of 10°C/min using nitrogen as a protective gas.

X-ray powder diffraction (XRPD)

XRPD patterns were recorded at ambient conditions with a STOE STADI P diffractometer (CuKα1 radiation, primary Ge-monochromator, position sensitive detector (PSD), 3° to 42° 2-theta angular range, 0.5° 2-theta PSD step width, 40 s per step measurement time). The samples were analyzed without further processing (e.g. grinding or sieving) of the material.

Single crystal X-ray diffraction

The crystal structure was obtained from synchrotron data (Swiss Light Source (SLS) synchrotron, PX II beamline). The structure was solved and refined with standard crystallographic software (ShelXTL from Bruker AXS,Karlsruhe).

In vitro Dissolution

The dissolution tests were conducted with a miniaturized USP-2 method (Sotax AT7 smart dissolution station) in 200 mL glass vessels. The dissolution experiments were carried out under non-sink conditions in triplicate with 1.5 mL TXM suspension (~6 mg TXM) in 150 ml simulated gastric fluid (SGF) pH 2 [2] at 25°C with freshly prepared TXM suspension and with suspension stored at 4°C for 1 week. The paddle speed was 25 rpm and the drug concentration was directly determined by UV online detection at 377 nm.

Ultra Performance Liquid Chromatography (UPLC)

Analysis was performed on a Waters Acquity™ system, equipped with an Acquity UPLC™ BEH C18 column (2.1x50 mm, 1.7 mm particles). A linear gradient with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) was run with 20% B to 100% B during 0.5 min, flow rate 0.75 ml/min, total run time 1.2 min, column temperature 30°C, UV detection at 377 nm.

Results

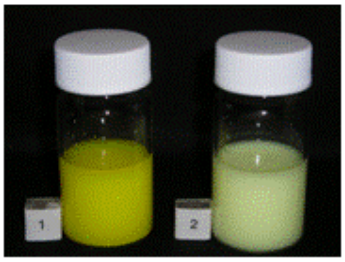


Figure 2. TXM suspension freshly prepared (1) and after storage for 1 week at 4°C (2).

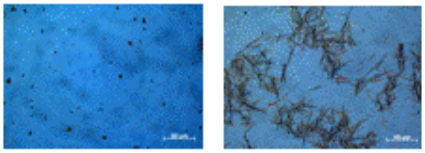


Figure 3. Photomicrographs of freshly prepared TXM suspension (left) and after storage for 1 week at 4°C (right).

Oral suspension formulations with TXM exhibited a visual change in color from yellow (freshly prepared suspension) to light yellow (after storage) (Fig. 2). Microscopic analysis revealed the formation of crystal needles (Fig. 3). Analysis of the isolated needles by XRPD and UPLC measurements indicated the formation of TXM cocrystals with methylparaben, a commonly used preservative in oral suspension formulations.

The novel TXM methylparaben (1:1) cocrystal phase was confirmed by its unique thermal and XRPD properties and by single crystal X-ray diffraction (Figs. 4-6). The in vitro dissolution of the suspension with TXM-methylparaben cocrystals (TXM suspension after storage) was significantly improved compared to the freshly prepared suspension with TXM (Fig 7).

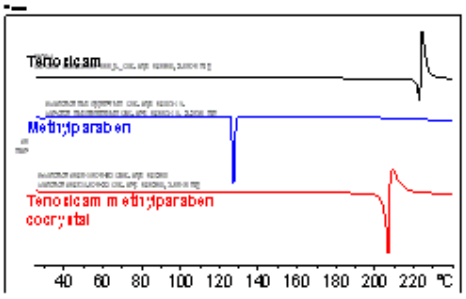
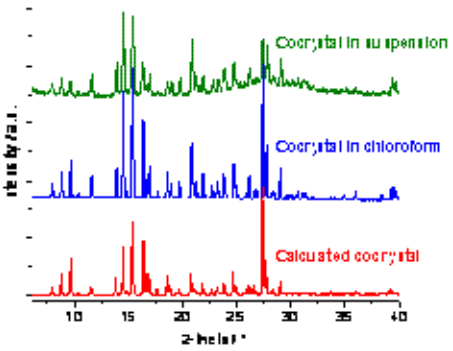


Figure 4. DSC curves of TXM (top), methylparaben (middle), and TXM methylparaben cocrystal (bottom).



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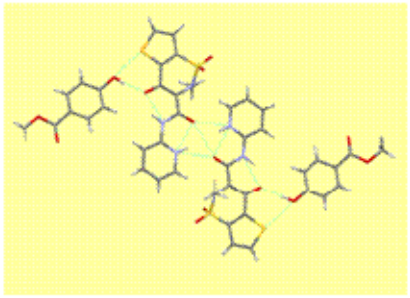

Figure 6. Crystal packing in TXM methylparaben cocrystal. Light blue dotted lines represent hydrogen bonds. The hydrogen bonding between drug and ligand consisted of an interaction between the hydroxy group of the methylparaben molecule and the pentacyclic sulfur as well as the deprotonated oxygen of TXM which is present in its zwitterionic form [3].

Table 1. Crystallographic data of the TXM cocrystal with methylparaben (1:1).

	TXM cocrystal with methylparaben (1:1)
Empirical formula	C23 H19 N3 O7 S2
Formula weight	489.53
Temperature [K]	50
Wavelength [Å]	0.710
Crystal system	Triclinic
Space group	P-1
a [Å]	8.0500 (16)
b [Å]	10.060 (2)
c [Å]	12.170 (2)
α [deg]	87.76 (3)
β [deg]	72.03 (3)
γ [deg]	74.66 (3)
Volume [Å³]	1045.1 (4)
Z	2
Calculated density [g/cm³]	1.555
Absorption coefficient [mm⁻¹]	0.305
F(000)	506
Crystal size	0.3 x 0.05 x 0.02 mm
Reflections collected	14432
No. of unique reflections	3758
R(int)	0.0571
Final R indices [I>2sigma(I)]	R1 = 0.0477, wR2 = 0.1327
R indices (all data)	R1 = 0.0612, wR2 = 0.1380
Goodness-of-fit on F^2	1.072
Data deposition	Roche CSD structure No. 1776

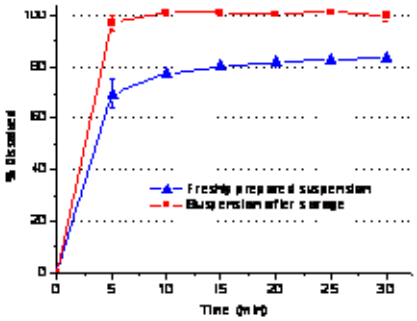

Figure 7. Dissolution profiles of freshly prepared TXM suspension and after conversion to TXM methylparaben cocrystals (i.e. after storage of the suspension for 1 week at 4 °C).

Conclusions

- New cocrystal of TXM with methylparaben identified in oral suspension formulation
- Cocrystal formation confirmed by polarization microscopy, TGA, DSC, UPLC, XRPD, and single crystal X-ray diffraction
- Cocrystallization of TXM with methylparaben was associated with an improvement of the in vitro dissolution behaviour of TXM
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References

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Conclusion of part 3

The present series of tests indicate that the small volume dissolution is a useful tool for the characterization of immediate release drug product and can be applied as well already in early phase development on API. By increasing the discriminating power of the dissolution method, it potentially improves know how about formulations on typical events which occur during pharmaceutical development as aging, dose change or scale up.

The accessories investigated in the scope of this third experimental part have very low cost, are easy to set up and can be used in most of the laboratory where a standard system already exists. It allows having a robust and reproducible system that can be particularly of interest in early development phases where during screening, the formulation is often developed for studies in animals and dissolution should be ideally conducted using media volumes in line with the animal physiology.

In addition is it to note that the ER4H and ER8H mentioned in the paper 3, correspond to the ER4H and Er8H investigated in the paper 2. It was shown that the change in hydrodynamics did not strongly impact the release rate of these tablets, in particular for the Er8H. This was expected since the main drivers of the release (CQA) was identified as the diffusion mechanism. The small dissolution equipment does then serve indirectly as well as a good confirmation of this hypothesis.

With this regard small volume dissolution is a method of choice in case of screening for critical quality attribute and represents a nice alternative to classical compendial pharmacopeia systems.

3.4. Experimental part 4

In sharp contrast to the branded pharmaceutical market, which has stalled in recent year, the generics market is enjoying a period of unprecedented success. In 2005 the world generics market was worth $45bn, a growth of 14% on the previous year. It is expected this level of growth to continue. The loss of patent protection by 2009 of almost $80bn worth of top selling drugs will be the major driving force for this generic market growth.

In the scope of this thesis, the question arose internally at Roche if dissolution can be a good tools to detect difference between Mycophenolate Mofetil (MMF) formulations.

The aim of this 4th experimental part was to compare the original brand CellCept® 500 mg tablets with generic products which are commercially available around the World using in vitro dissolution testing. In addition to the ANDA method, investigation were undertaken to simulate in vitro the difference in performance due for instance to food intake or to highlight any other difference in view of in vitro performance.

For the same API, differences in solid state properties, formulation and / or manufacturing can lead to differences in bioavailability from one finished product to another. As the in vivo drug dissolution is the rate-limiting factor in drug absorption, for BCS class II drugs, the use of appropriate designed in vitro dissolution tests can potentially discriminate between formulations with different bioavailability. Different kind of dissolution methods were investigated based on the physico-chemical properties of the MMF and the data are presented in paper 4 and its supplements.

The paper 4 entitled **"Mycophenolate mofetil: use of simple dissolution technique to assess difference between innovator and generic formulations" "** was submitted to Pharmaceutical Technologies.

The data are also well abstracted in a poster entitled "***In vitro* dissolution of mycophenolate mofetil: comparison between innovator and generic formulations** "which was presented twice. At BPS Winter meeting 2008, Abstract 0225 and at the ACCP/ESCP International Congress 2009, Presentation 114E.

In conclusion of these investigations, important differences exist between the different generic formulations with regard to *in vitro* performance.

In a next step, an exploratory clinical testing was set up to evaluate the pharmacokinetics of different generics that showed the more pronounced difference. The data are presented as a supplement 1 to paper 4 entitled "**Confirmation of the hypothesis in human** "

All the studies and the importance of having a strong discriminating dissolution methods, IVIVC/R and QbD as well as the general need of having a strategy for brand protection for all drugs already in the early development is discussed shortly in the conclusion of this 4th parts of the thesis.

This effort was made by Roche in order to have brand names of products not associated with therapeutic failure.

Original Article

Mycophenolate mofetil: use of simple dissolution technique to assess difference between generic formulations

E. Scheubel[1,2 *], L. Adamy[2] and JM. Cardot[1]

[1] Biopharmaceutical department, Faculty of Pharmacy, University of Auvergne
28 Place H. Dunant, BP 38, 63001 Clermont-Ferrand France
[2] F. Hoffmann-La Roche Ltd. Pharmaceutical Division, Galenical & Analytical Development. CH-4070, Basel, Switzerland

*Author to whom correspondence should be addressed *e.mail: emmanuel.scheubel@roche.com Tel: +41616886357 ; Fax +41616870280.*

Abstract

Purpose: Mycophenolate mofetil (MMF) is an immunosuppressive agent indicated for the prophylaxis of acute rejection in patients receiving allogeneic renal, cardiac or hepatic transplants. It's a Biopharmaceutics Classification System class II substance that has a strong pH-dependent solubility profile. Consequently, differences in solid-state properties, formulation and/or manufacturing processes of MMF can lead to disparities in bioavailability between brands of the same drug. This study was conducted to compare the *in vitro* dissolution profile of the original MMF innovator brand (CellCept*, Roche) with available generic products. **Methods:** Two representative batches of CellCept 500 mg tablets and 14 different generic formulations were tested using different dissolution testing scenarios simulating conditions in the proximal gastrointestinal tract. These scenarios took into account stomach and/or small intestine media composition, surface tension, pH, increased buffer capacity and osmolarity after food intake. **Results:** Eight of the generic formulations tested passed the quality control dissolution test (pH 1.1) according to specification Q=75% after 5 min (i.e. all single units >80% dissolved), and 12 passed the specification Q=85% after 15 min (i.e. all single units >90% dissolved). This suggests an almost homogenous dissolution rate in an acidic environment between formulations. However, at pH 4.5, large variations in *in vitro* dissolution performance between generic formulations were observed (extremes resulting in more than 60% dissolved difference after 30 mins). Marked variability was seen among the different generic formulations and between the innovator brand, CellCept.

Conclusion: Important differences exist between the different generic formulations with regard to *in vitro* performance. As MMF is required for life-long use, changes in drug performance as a result of switching between formulations may have serious clinical consequences (e.g. organ rejection). Therefore, clinical testing is necessary to evaluate the pharmacokinetics and the impact on clinical safety of a switch between brands.

** CellCept is a registered trade name of Roche Products Ltd.*

Key words

Mycophenolate mofetil, generics, dissolution in vitro, switchability.

Short title

Use of dissolution to assess difference between generic formulations

Previous presentation

An abstract of this original paper has been presented at British Pharmacological Society (BPS) Winter Meeting 2008, Abstract 0225.

Introduction

After patent protection of original brand is expired, healthcare systems are encouraging the use of generic medicines. While the economic need to limit healthcare costs using generics is not questioned, it is important to ensure that patient health is not compromised. Equivalence has to be shown usually based on bioequivalence on healthy volunteers[1]. However, as generic products are approved based on comparison with the innovator only[2], one could argue that switching from one generic product to another might give rise to complications due to the potentially greater disparity between two generic products than between any single generic product and the innovator. In particular switch from innovator to generic for life long treatment like immunosuppressive prescription may be of consequence[3,4].

Mycophenolate Mofetil (MMF) is an immunosuppressive agent indicated for the prophylaxis of acute rejection in adult recipients of renal, cardiac or hepatic transplants as well as paediatric recipients of renal transplants. MMF is currently being prescribed for life long use. MMF is a weak base classified as a BCS class II[5] substance, exhibiting a strong pH dependent solubility profile (solubility decreases when pH increases). It is absorbed rapidly and hydrolyzed by esterases to the active metabolite mycophenolic acid (MPA)[6]. The maximum of absorption (Cmax) in man is observed after approx 30 minutes in fasted state

For the same API, differences in solid state properties, formulation, excipients and or manufacturing can lead to differences in bioavailability from one finished product to another[7]. As the in vivo drug dissolution can be the rate-limiting factor in drug absorption for BCS class II[8] drugs, the use of appropriate designed in vitro dissolution tests can potentially discriminate between formulations with different bioavailability. In case of Mycophenolate Mofetil (MMF), differences in dissolution profiles can potentially be useful predictors of clinical differences[9], since the absorption of this drug with a very short Tmax in fasted state is limited by the dissolution rate.

The aim of this study was to compare, using classical in vitro dissolution testing, the original brand CellCept 500 mg tablets with generic drug products which were commercially available around the world in 2008. In addition to the registered NDA[10] (QC) method, investigations were undertaken to simulate *in vitro* various conditions which are accounted in vivo.

Material and methods:

Material

Egg lecithin (E PC S, purity >96%) was obtained from Lipoid (Ludwigshafen, Germany) and sodium taurocholate (NaTC, 97% pure) was used as received from Prodotti Chimici e Alimentari SpA, Basaluzzo, Italy.

Phosphate buffer, sodium chloride (ACS), 37% hydrochloric acid (fumed), 85% ortho-phosphoric acid, ethanol (99.9%), acetic acid as well as HPLC grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Water was obtained from a Milli-Q (Millipore, Milford, MA, USA) water purification system. Commercial milk (UHT) with at least 3.5% fat was used.

Two representative marketed batches of Roche CellCept 500 mg tablet and 14 MMF generic samples from different origins other the world were tested (please note that the generics were purchased in 2008 from countries where patent was expired.)

Method

The dissolution profiles were conducted using a Sotax AT7 smart apparatus (Sotax, Allschwill, CH). In addition to the suggested ANDAs method for release (Paddle 50 rpm in HCL[10]), different media taking into account stomach and or small intestine media composition, surface tension, pH, increased buffer capacity, osmolarity and pH change after food intake were investigated[11]. The different working conditions are presented in Table 1.

Paddle speed was set at 50 rpm and temperature at 37°C, with n=3 to 12 units depending on the working conditions or the screening activity. Samples were withdrawn at predefined time

intervals from each vessel and not replaced. Sampling was automated and dissolution samples were directly filtered and subsequently measured by a validated UV detection method.

The solubility of the MMF was assessed in each dissolution medium mentioned in table 1. Duplicate samples were incubated with an excess of compound in a 10 ml volumetric flask at 37°C with constant rotation for 4 hours and the dissolved quantity was measured with a validated HPLC-UV detection method.

To simulate the impact of the pH variations or medium composition on the dissolution behavior of the 500 mg MMF tablets, the comparisons listed in Table 2 were investigated. The dissolution profiles were measured independently in each media according to Table 1. For this first screening only two generics, which have shown the highest difference with regard to performance using the NDA method were investigated in comparison to CellCept 500 mg.

For comparison of the dissolution profiles, the curves were estimated equivalent in HCl if they fulfilled the ANDAs specification, The comparison of various CellCept batches allows to fix the limits at Q=75% after 5 minutes [i.e. Stage 1, all single units greater than 80% dissolved] and Q= 85% after 15 minutes [i.e. Stage 1, all single units greater than 90% dissolved]. This first selection step will allow selecting the formulations studied afterward: a generic fulfilling the requirements and one that does not pass the requirements.

In the other tested media, the similarity factor f2[12] was calculated for each MMF generic versus CellCept, where applicable (at least 3 points in the ascending part of the curve with maximum one above 85%). The FDA[13] and EMEA[14] recommended that two dissolution profiles are similar if f2 is between 50 and 100.

Table 1: Dissolution in vitro working conditions used for screening of MMF

Medium	**pH**	**Volume**	**n=**	**comments**
HCL 0.1 N	1.1	900 ml	12	QC method, pH of fasted state stomach
Acetate buffer 0.05 M	4.5	900 ml	6	pH of fed state stomach
FaSSIF	6.5	500 ml	3	Fasted State Simulated Intestinal Fluid
FeSSIF	5.0	500 ml	3	Fed State Simulated Intestinal Fluid
FeSSGF	5.0	500 ml	3	Fed State Simulated Gastric Fluid

Table 2: Overview of the in vitro methods simulating change after food intake.

Test#	Simulated food intake	Compared media and pH	comments
1	Stomach	HCL pH 1.2 FeSSGF pH 5.0	Gastric pH decreases continuously after meal ingestion from pH 6.4 to 2.7. Middle condition was chosen.
2	Small intestine	FaSSIF pH 6.5 FeSSIF pH 5.0	To simulate pre and postprandial bile secretion, in upper small intestine before and after food intake
3	pH variation in stomach	HCL pH 1.1 Acetate pH 4.5	pH 4.5 was chosen as non sink method, allowing to dissolve 1 tablet 500 mg into 900 ml.

Results:

Solubility

Table 3 summaries the results of the solubility study, sink conditions being reached only in HCl 0.1N.

Dissolution

The dissolution of the various formulations in HCl 0.1N are presented in Figure 1. The Table 4 presents the main results according to ANDA specifications.

Four generics over 14 did not pass the S1 ANDA requirements (28%) and 2 out of 14 are out of specifications (OOS) according to the ANDA method after 15 minutes (14%). Generic 2 which was the more close to reference formulation and generic 4 that dramatically failed were selected for further investigations to select a media alternative to HCl 0.1N.

The Table 5 and figures 2 to 5 summarized the finding observed in various media.

A similar rank order was observed in all media for the three formulations, generic 2 being always equivalent to reference and generic 4 being always lower that reference. Figure 6 and Table 6 summarized the differences observed in acetate buffer (the simplest media that emphasizes differences). The dissolution under non strictly sink conditions could be questionable but highlights differences that could have a clinical relevance.

Table 3: Solubility of MMF in different media according to in vitro working conditions.

Medium	Solubility of MMF
FeSSGF pH 5.0	*290* mg/500 mL
FaSSIF pH 6.5 *Blank FaSSIF*	38 mg/500 mL *35* mg/500 mL
FeSSIF pH 5.0 *Blank FeSSIF*	548 mg/500 mL *324* mg/500 mL
HCL 0.1N pH 1.1	*4270* mg/L
Acetate pH 4.5	*600* mg/L

Table 4: Dissolution results according to NDA method

	CellCept		generic #														
sample ID#	1	2	1	2	3-A	3-B	4	5	6	7	8	9	10	11	12	13	14
Result in HCl 0.1N at 5'	S1	S1	OOS	S1	S2	S1	OOS	S1	OOS	S1	OOS	OOS	OOS	S2	S2	S1	S1
Result in HCl 0.1N at 15'	S1	S1	S2	S1	S1	S1	OOS	S1	S2	OOS	S1	S2	S1	S1	S1	S2	S1

S1, S2 correspond to the USP stage of acceptance according to USP <711>. OOS means Out Of Specification.

Table 5: Comparison of dissolution profiles using similarity factor f2 in various media

Medium	Cellcept 2	generic 2	generic 4
FaSSIF	*	*	*
FeSSIF	ref	71	19
FeSSGF	ref	60	29
HCl	ref	**	26
Acetate	ref	71	15

* all three profiles lie under 10 % release and are super imposable

** not applicable but profile are super imposable

Table 6: Dissolution results according to acetate method.

	CellCept		generic #														
sample ID#	1	2	1	2	3-A	3-B	4	5	6	7	8	9	10	11	12	13	14
f2 in acetate	81	ref	52	71	47	83	15	26	37	23	24	36	53	67	89	73	25
Verdict	P	P	P	P	F	P	F	F	F	F	F	F	P	P	P	P	F

P correspond to the Pass and F corresponds to fail the f2 acceptance criteria

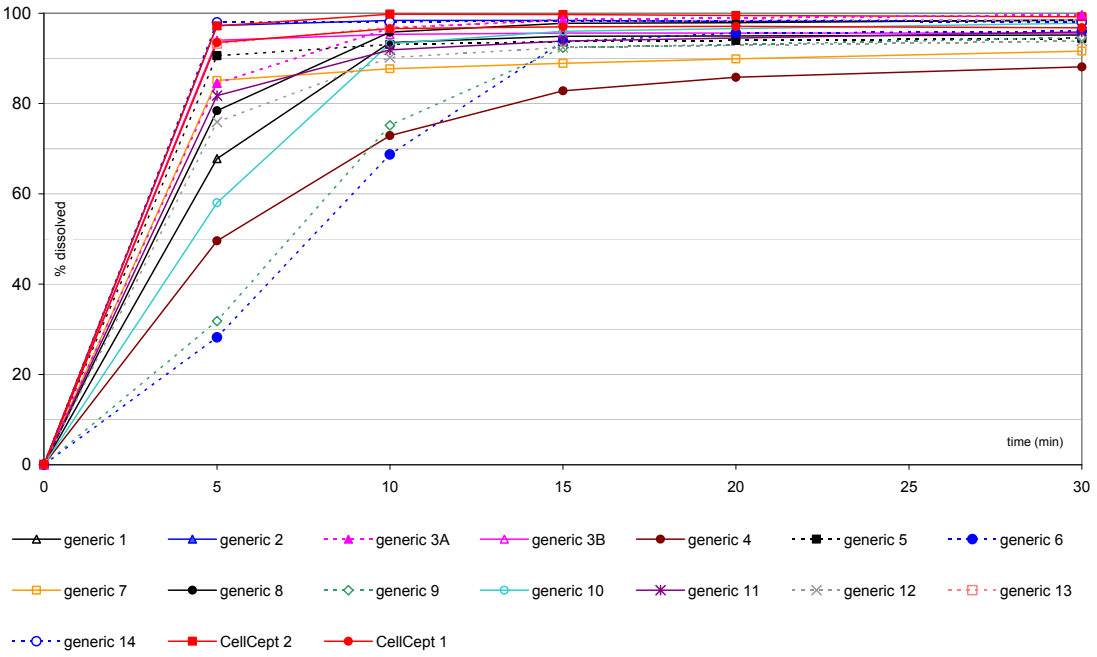

Figure 1: Dissolution profiles according to NDA method. Roche CellCept® are in red (n=12), the standard deviation after 5 minutes lies between 2 % to maximum 8 % and after 15 minutes at maximum 2%. The observed variations within the tested tablets batches are very low

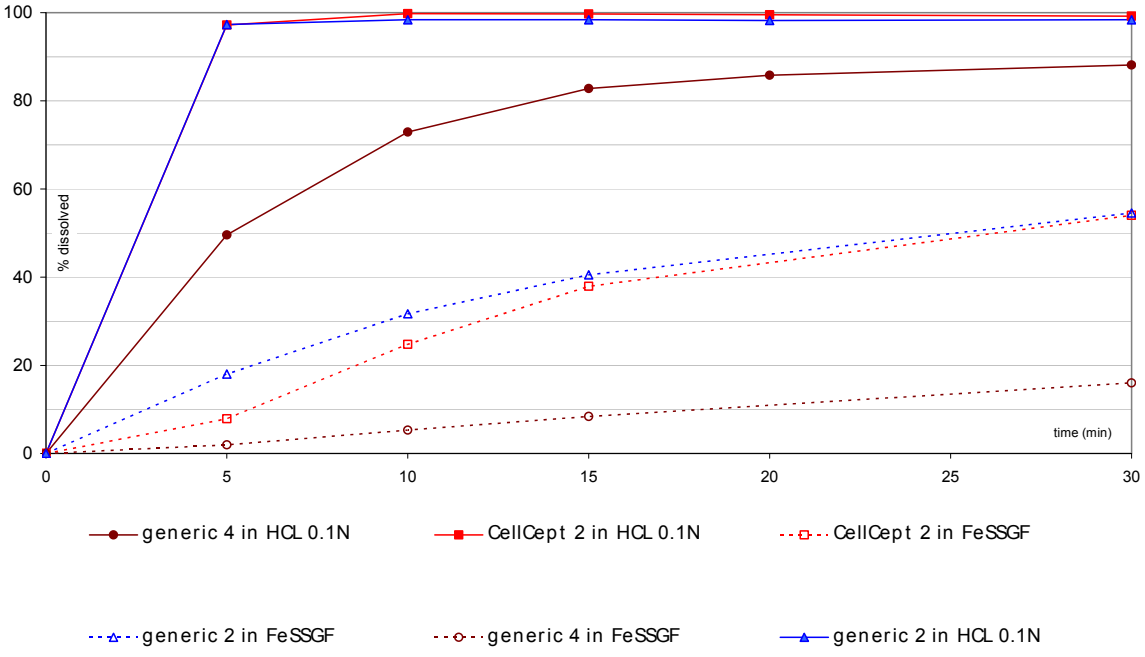

Figure 2: Test#1: Dissolution profiles of CellCept® (red) and 2 generics in media simulating fasting state (HCL 0.1N) and Fed state (FeSSGF pH 5.0, dotted line) in stomach. The standard deviation after 15 minutes lies at maximum 2%. The observed variations within the tested tablets batches are very low.

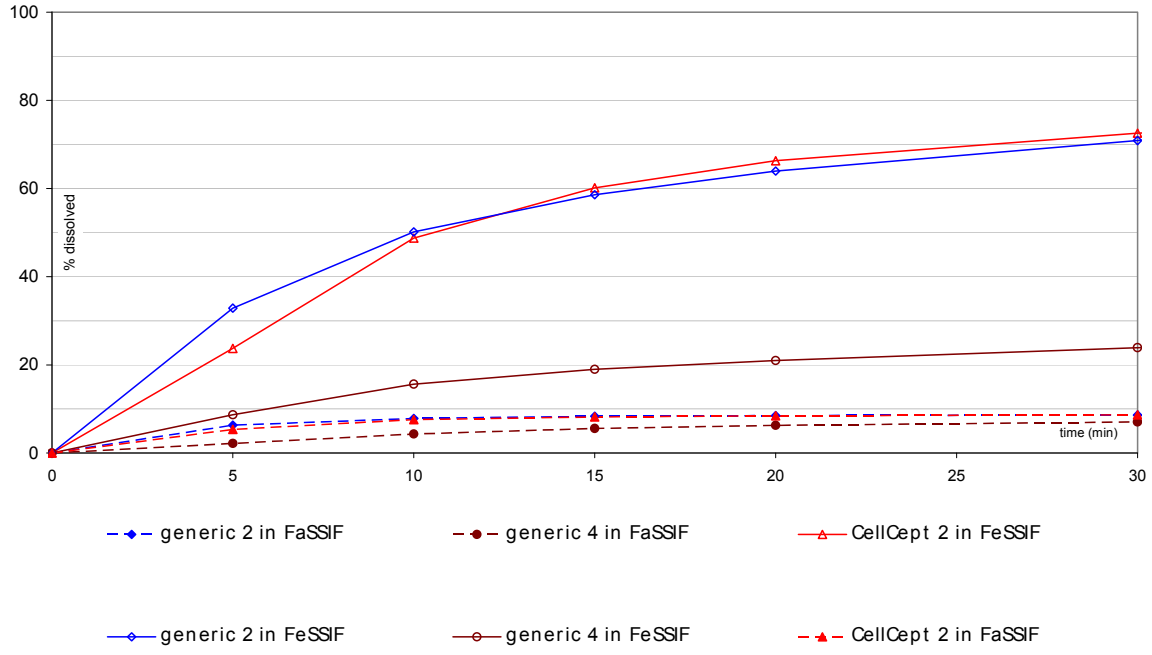

Figure 3: Test#2 : Dissolution profiles of CellCept® (red) and 2 generics in media simulating fasting state (FaSSIF, dotted line) and Fed state (FeSSIF) in small intestine.
The standard deviation after 15 minutes lies at maximum 2%. The observed variations within the tested tablets batches are very low.

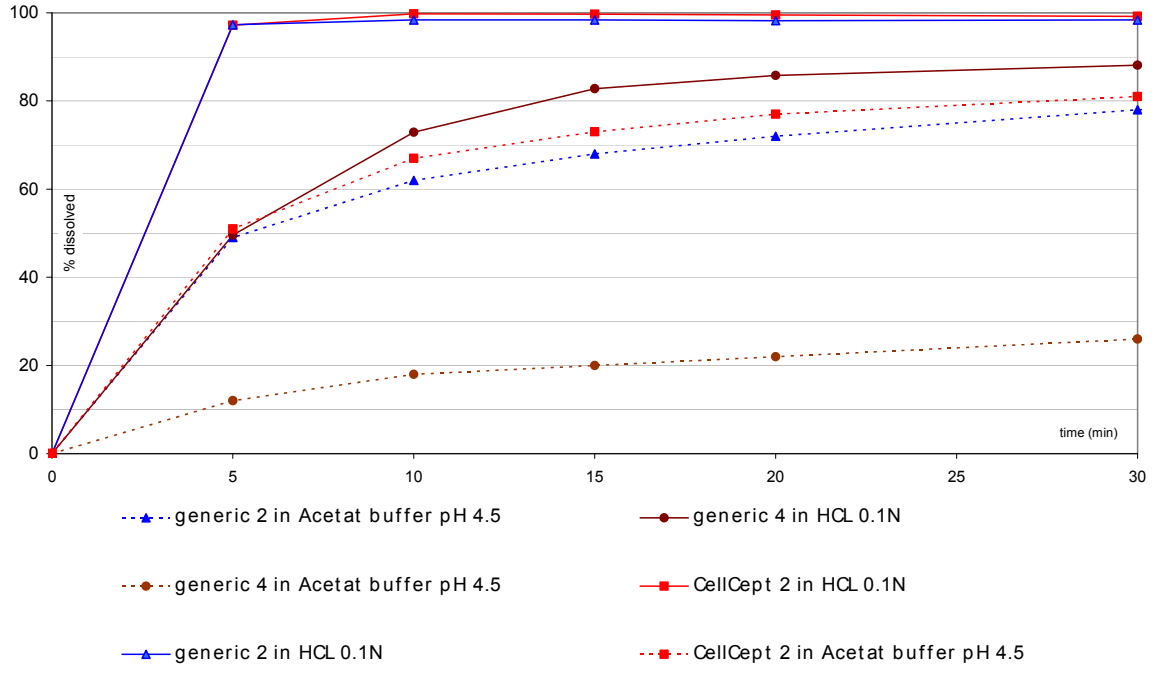

Figure 4: Test#3: Dissolution profiles of CellCept® (red) and 2 generics in media simulating pH variation in stomach: HCL 0.1N and Acetate pH 4.5 (dotted line).

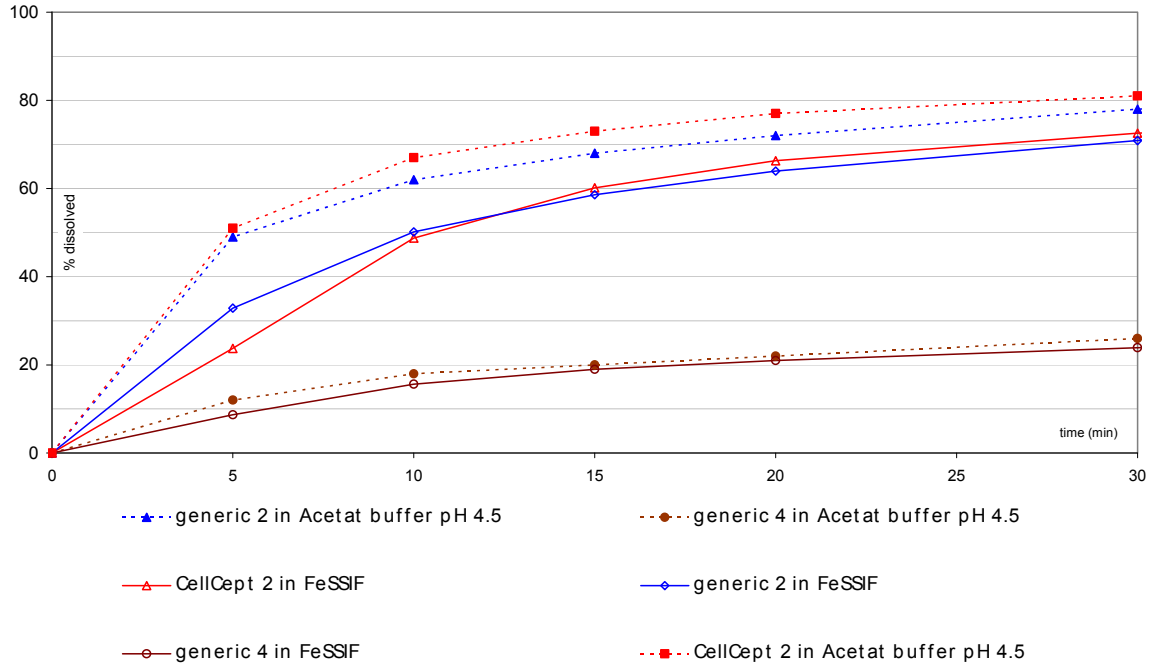

Figure 5: Dissolution profiles of CellCept® (red) and 2 generics in acetate pH 4.5 (dotted line) and FeSSIF pH 5.0.

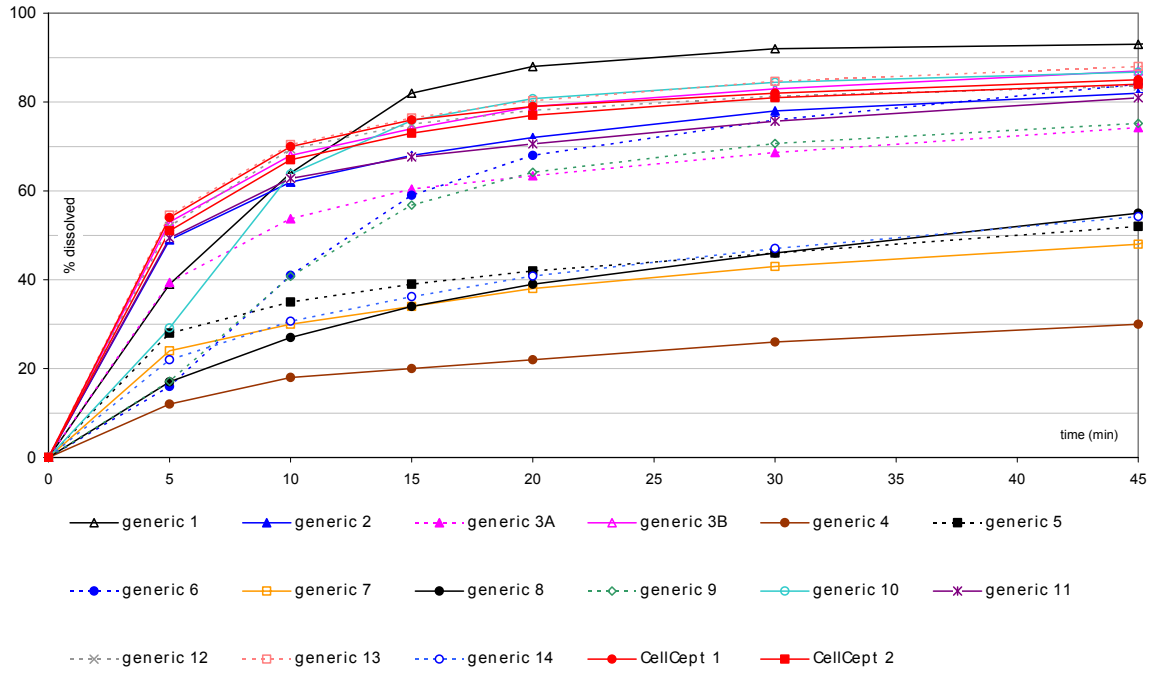

Figure 6: Dissolution profiles of all tested generics at pH 4.5. Roche CellCept® are in red. The standard deviation after 5 minutes lie at maximum 5 % and after 15 minutes at maximum 2% and after 45 minutes at 2 %.

Discussion:

Mycophenolate Mofetil exhibited a typical pH dependent solubility profile. Sink condition (3 times saturation in dissolution volume) are fulfilled only in HCL 0.1N. In the 900 ml of acetate buffer pH 4.5 the entire tablet dose can be dissolved. Comparison of the solubility of the blank FaSSIF and blank FeSSIF versus FaSSIF and FeSSIF respectively suggested that the impact of bile salt and lecithin were well pronounced and consequently food intake can impact the solubility of MMF in vivo.

According to the ANDA method (see figure 1), different profiles for the generics in comparison to the reference CellCept 500 mg can be observed. 9 out of 14 tested generics have successfully passed the ANDA dissolution test (see table 1) according to our current specification Q=75% after 5 minutes and 12 have successfully passed according to the specification Q= 85% after 15 minutes. Four of them only fulfilled the acceptance criteria after USP stage 2 and 2 out of the 14 were out of our current specifications. These results suggest an almost homogenous dissolution rate in acidic environment even if at least 2 formulations are not complying with the dissolution specifications of FDA suggesting that they could not be on the market in US. Due to the limited amount of available tablets for each generic, two generics (2 and 4) representing the highest and the lowest value found after 15 minutes with the NDA method, were selected for dissolution method screening. The impact of food intake and or pH change on MMF dissolution performance was evaluated by comparison of the obtained profiles measured in different media independently as mentioned in table 2.

The simulation of pH change and impact of medium composition in the stomach as observed in figure 2 (test#1), suggested similar impact of dissolution media for both CellCept and tested generics (i.e. after 15 minutes decrease of approx 60 % from HCL to FeSSGF) the ranking observed in HCl 0.1N being respected. The similarity factor f2 confirmed that generic 4 is not comparable to CellCept whereas generic 2 resulted in similar profile (see table 5).

The impact of pH change and medium composition in upper small intestine before and after food intake, to simulate pre and postprandial bile secretion, as simulated in figure 3 (test#2), suggested similar behavior for CellCept and generic 2 (e.g. after 15 minutes decrease of approx. 50 % from FeSSIF to FaSSIF). Generic 4 showed a significant lower profile in FeSSIF and consequently a less pronounced decrease in FaSSIF. In fact at pH 6.5 (FaSSIF), the maximum solubility is reached (37.5 mg in 500 ml), that resulted in a plateau at approx 7 - 8 % for all tested samples. At pH 5.0 (FeSSIF) conditions in which the complete dose can theoretically be solubilized (500 mg in 500 ml), a significant difference in performance for generic 2 and 4 is observed. The f2 factor being significantly lower than 50 for generic 4.

Simulation of pH change was further investigated by using common dissolution media as figured out in figure 4 (test#3). Generic 2 showed a decrease of approx. 30 %, slightly greater than CellCept, whereas generic 4 resulted in 20 % dissolved only after 15 minutes. The f2 factor being the lowest observed in table 6 for generic 4.

Based on this screening, the highest discriminatory power is observed with FeSSIF pH 5.0 and acetate buffer pH 4.5. At these pH's and with the working conditions used, comparable solubility exists and similar profiles can be observed (see figure 5). In practice, the acetate buffer is advantageous since it is simple to prepare and in comparison to more complex approaches using simulated intestinal fluids as often seen in literature reports [15,16,17] , it has the potential to serve both as a robust dissolution method and a biorelevant method with a high discrimination power. This method was therefore set up for further investigations on the available generics.

Large variations of in vitro dissolution performance from generic to generic were observed (see figure 6 and table 6). The acetate pH 4.5 method identified clearly two levels of performance in vitro from 15 minutes onwards. Extremes resulting in less than 50% dissolved after 30 minutes for 4 generics and less than 30 % dissolved for one generic out of the 14 tested variants. Significant inter batches variability (generic 3A and 3B) was also found among generics in

comparison to CellCept tablets 500 mg (CellCept 1 and 2 resulted in very similar profiles with f2 = 82).

PH 4.5 being a natural pH in fasting state of the stomach[18], a direct consequence of this marked difference of performance at this pH could result in strong decrease of release rate for some generics whereas CellCept tablets and other generics remain fast dissolving tablet (> 80 % after 30 minutes). Under fasting conditions, release of MMF in patients having high variation of gastric pH or achloridia could be directly impacted depending on the generics quality. As well high performance variations in fed state stomach (with pH from 6 to 3[11]) can then be anticipated. In addition, with a drug having a Tmax of approx 30 minutes, the difference observed on figure 6 for some generics would result in significant delay of the absorption kinetic (Tmax and Cmax) at higher pH since the drug is potentially not completely dissolved at gastric emptying (after 15-30 minutes in fasting conditions). It can be clearly shown that different qualities of generics exist and that variable performance can be associated from generic to generic even if the therapeutic impact could not be anticipated from those results, a special attention should be given to the generics with the lower results. As generic drugs are approved based on comparison with the innovator brand only, it is possible that switching between generic products may lead to greater disparity than switching between a single generic product and the innovator brand – with unknown clinical consequences. In view of these uncertainties, further research should be done to evaluate the pharmacokinetic and safety profiles of generics with lower dissolution results and to determine if they are all therapeutically equivalent. In the case of MMF, change in performance can dramatically affect the risk associated to the medication (e.g.: acute rejection in patients).

The notions developed in the US FDA draft Guidance for Industry; Average, Population, and Individual Approaches to Establishing Bioequivalence, Aug 1999[19] (which was never adopted) defined the two interesting concepts of switchability and prescribability could be of interest for typical drugs like anti rejection drugs. Prescribability refers to the clinical setting in which a

practitioner prescribes a drug product to a patient for the first time. In this setting, the prescriber relies on an understanding that the average performance of the drug product has been well characterized and relates in some definable way to the safety and efficacy information from clinical trials.

Switchability refers to the setting in which a practitioner transfers a patient from one drug product to another. This situation arises with generic substitution, as well as with certain post approval changes by an innovator or generic firm in the formulation and/or manufacture of a drug product. Under these circumstances, the prescriber and patient should be assured that the newly administered drug product will yield comparable safety and efficacy to that of the product for which it is being substituted.

In our case MMF could be considered as a prescribability drug. These investigations highlight the importance of developing discriminating dissolution methods, taking into account the physico-chemical properties of the drug as well as the characteristic of the formulation. The methods do not necessarily require a complex setting; they need to match the key parameter likely to impact the performance. The results reported here were generated with a limited number of tablets from only one or two lots of each generics or manufacturer. It is unknown whether these results are representative for other lots. Nevertheless, huge differences were observed some of them being even out of specifications using the current method proposed by NDA. The acetate buffer media highlights the differences and is able simply to discriminate between batches that fully comply in HCL. Acetate pH 4.5 method discriminated 8 variants out of 14 whereas HCL method identified 2 OOS variants after 15 minutes. Considering the nature of the drug, a bioequivalence study is mandatory according to FDA note of guidance (Guidance for Industry Bioavailability and Bioequivalence Studies for Orally Administered Drug Products; General Considerations 03/03) as a therapeutic failure could not be allowed. Anyway a simple dissolution using a standard acetate buffer medium could help to insure a greater quality of generic development and quality control that insure a better efficacy and safety.

Conclusion:

In summary the generics and CellCept tablets 500 mg dissolution profiles obtained with an alternate dissolution method, suggest that important differences may exist between the different generics with regard to in vitro performance. In case of Mycophenolate Mofetil (MMF), differences in dissolution profiles can potentially be useful predictors of clinical differences, since the absorption of this drug with a very short Tmax in fasted state is limited by the dissolution rate. Taking into account that MMF is currently being prescribed for life long use, additional clinical testing may be necessary to evaluate the pharmacokinetics and clinical safety impact of a switch from one generic quality to another.

If these in vitro findings are confirmed by clinical studies, and significant implications for safety and effectiveness are proven, they should be considered by clinicians to potentially safeguard patients who choose to purchase generics drugs. In this case, MMF formulations should be similar in HCL as well as in acetate pH 4.5 before being further developed by new formulators. This prior in vitro knowledge should be leveraged as a resource to aid in the development, justification of tests and specifications for new products and ensure safety and similarity of generic for the patients.

Transparency

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Declaration of financial/other relationships. Both authors Emmanuel Scheubel and Laurent Adamy are employed by Roche Basel, Switzerland. There is no competitive interest

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The data were abstracted in form of a poster entitled "***In vitro* dissolution of mycophenolate mofetil: comparison between innovator and generic formulations** "which was presented twice. At BPS Winter meeting 2008, Abstract 0225 and at the ACCP/ESCP International Congress 2009, Presentation 114E.

The poster is presented enclosed, ones completed and ones enlarged in 3 parts dor a better visibility.

In vitro dissolution of mycophenolate mofetil: differences observed between innovator and generic formulations

Emmanuel Scheubel, Laurent Adamy
F. Hoffmann-La Roche Ltd, Basel, Switzerland

INTRODUCTION

- Mycophenolate mofetil (MMF) is an immunosuppressive agent indicated for the prophylaxis of acute rejection in patients receiving allogeneic renal, cardiac or hepatic transplants. MMF, a weak base, is a Biopharmaceutics Classification System (BCS) class II substance,[1] exhibiting a strong pH-dependent solubility profile. It is absorbed rapidly and hydrolysed by esterases to the active metabolite mycophenolic acid. In humans, time to maximal mycophenolic acid concentration (t_{max}) is about 30 minutes.
- Differences in solid-state properties, formulation and/or manufacturing processes can lead to disparities in bioavailability between brands of the same drug. As generic drugs are approved based on comparison with the innovator brand only, it is possible that switching between generic products may lead to greater disparity than switching between a single generic product and the innovator brand – with unknown clinical consequences.
- The *in vivo* drug dissolution is a rate-limiting factor in drug absorption for BCS class II drugs,[2] meaning the use of appropriately designed *in vitro* dissolution tests can potentially discriminate between formulations with different bioavailability. This study was undertaken to compare the *in vitro* dissolution of the original MMF innovator brand (CellCept®, F. Hoffmann-La Roche Ltd) with available generic products.

MATERIALS AND METHODS

- Two representative batches of CellCept® 500 mg tablets and 14 different approved generic formulations were tested using different dissolution testing scenarios simulating conditions in the proximal gastrointestinal tract. These scenarios included stomach and/or small intestine media composition, surface tension, pH, increased buffer capacity and osmolarity after food intake.

RESULTS

- Eight of the 14 generic formulations tested passed the quality control dissolution test (pH 1.1) according to specification Q=75% after 5 minutes (i.e. all single units >80% dissolved), and 12 passed the specification Q=85% after 15 minutes (i.e. all single units >90% dissolved) [fig 1]. This suggests an almost homogenous dissolution rate between brands in an acidic environment.

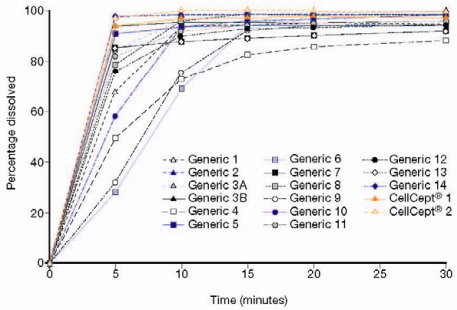

Figure 1. Dissolution profiles of CellCept® (orange) and generic MMF formulations at pH 1.1.

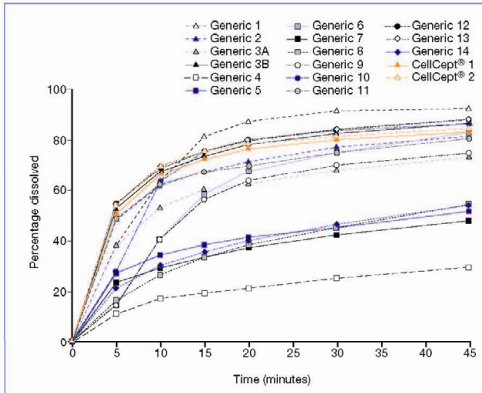

Figure 2. Dissolution profiles of CellCept® (orange) and generic MMF formulations at pH 4.5.

- However, at pH 4.5, large variations in *in vitro* dissolution performance between generic formulations were observed (extremes resulting in more than 60% dissolved difference after 30 minutes) [fig 2]. Marked variability was seen among the different generic formulations and between the various generic formulations and CellCept®.

DISCUSSION

- The natural variation in gastric pH in the fasted state (pH 1.0 to 4.5)[3] may result in a strong decrease in the release rate for some generic formulations that were tested. This may significantly delay t_{max} and reduce maximal absorption at higher pH levels because the drug may potentially not have been completely dissolved before gastric emptying occurs (typically 15–30 minutes under fasting conditions).
- It should be noted that these results were obtained using tablets from only one or two lots from each manufacturer. It is unknown whether these results are representative of other lots. Nevertheless, large differences were noted between some generic formulations and CellCept®.

CONCLUSION

- Important differences exist between the different generic formulations with regard to *in vitro* performance. As MMF is required for lifelong use, changes in drug performance as a result of switching between formulations may have serious clinical consequences (e.g. organ rejection). Therefore, clinical testing is necessary to evaluate the pharmacokinetics and the impact on clinical safety of a switch between brands.

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Acknowledgements

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ACCP/ESCP International Congress 2009, presentation no. 114E

1

The poster was enlarged in three parts for a better visibility

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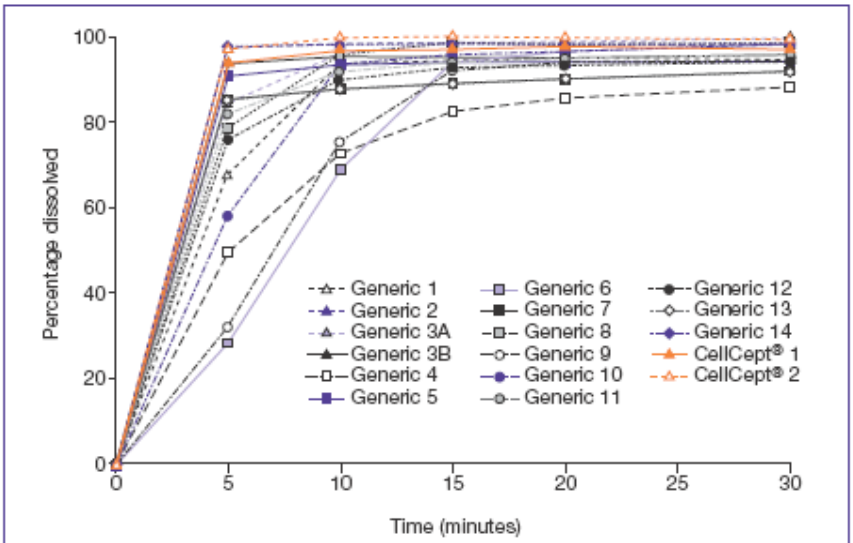

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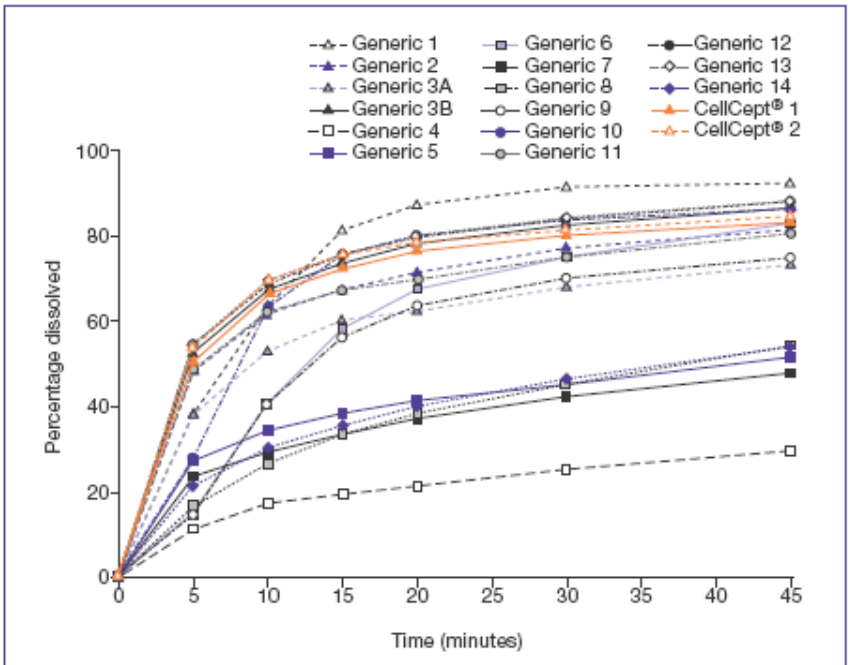

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Acknowledgements

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Mycophenolate mofetil: use of simple dissolution technique to assess difference between innovator and generic formulations.

Supplement 1 : confirmation of the hypothesis on human

Introduction

CellCept is available as a sterile powder for intravenous administration and as a powder for oral suspension, 250 mg capsules, and 500 mg tablets for oral administration. Recently, generic capsule and tablet formulations of MMF have received regulatory approval in various countries. Approvals have been based on demonstration of bioequivalence between each individual generic product and the originator product (CellCept) but there are no data available on the relative performance of generic formulations against each other. In order to confirm the difference observed in vitro with the proposed dissolution method at pH 4.5 as showed in paper 4, an exploratory study was intended to compare the pharmacokinetics of mycophenolate mofetil (MMF) from selected tablet formulations in healthy subjects as PoC.

Due to some IP limitations, the name of the generic are blinded and only some of the data are presented. The purpose of the study was to explore if the difference observed in vitro are relevant in vivo for three of the generics. CellCept tablets were included in the study for reference.

This approach shows the importance of in vitro in vivo relationship (IVIVR) for the determination of mycophenolate mofetil (MMF) performance and highlights the general need of having a strategy for brand protection for all drugs already in the early development by developing discriminating dissolution methods. The approach proposed to assess the quality of a product via a simple in vitro dissolution and so help companies to propose high quality products.

Material and Method

The three generics were selected out of 14 MMF generics based on the dissolution behavior in the discriminating medium explored in the paper 4. The three generics are. A representative marketed batch of CellCept® 500 mg tablet (F. Hoffman-La Roche Ltd) and MMF generic's 1-5-8 (please note that the generics were purchased in 2008 from countries where patent was expired). All study drug supplies were stored and handled according to the instructions on the product labelling.

In vivo bioavailability, bioequivalence assessment

An exploratory study to compare the pharmacokinetics of mycophenolate mofetil (MMF) from four 500 mg tablet formulations in healthy subjects was set up.

While it is recommended that CellCept be administered on an empty stomach in vitro dissolution data suggest that differences in formulation performance are greatest in media mimicking fed conditions. Therefore, to maximize the likelihood of being able to detect a difference between formulations in vivo, drug administration in this study were done with food. It was a randomized, open label, four treatments, four periods, four sequence, four-way crossover design and was conducted at a single centre. In each treatment period, subjects received a single 500 mg oral dose of MMF from one of four possible MMF tablet formulations with a high fat, high calorie meal.

In each period, blood samples for measurement of mycophenolic acid (MPA), the active metabolite, plasma concentrations were collected before (-0.5 h) and 0.33, 0.66, 1, 1.33, 1.66, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, 24, 36 and 48 h after drug administration. A total of 32 subjects were enrolled to ensure providing evaluable pharmacokinetic data.

Criteria for bioequivalence (ie, upper and lower 90% CIs within 0.80–1.25 for both Cmax and AUC) for one generic against other generic tablets were evaluated (FDA 2003).
The geometric means and associated two-sided 90% confidence intervals (CIs) were calculated by ANOVA for comparisons of the three generic formulations between each other (i.e. generic 8 vs. generic 5, generic 5 vs. generic 1 and generic 8 vs. generic 1). CellCept was monitored as reference.

In Vivo-In Vitro Correlation

Various approaches of relationship between in vitro and in vivo data based either on values (IVIVC) or rank (IVIVR), were tried on the main bioavailability parameters (Cmax and AUC**)** Linear regressions were primary sought. Calculations were performed with Microsoft Excel©.

Results

The dissolution in vitro profiles in acetate buffer pH 4.5 using paddle at 50 rpm are presented figure 1. CellCept and generic 1 showed similar performance with fast dissolving tablet with a plateau after 15 minutes and more than 85 % dissolved whereas generic 5 and 8 exhibited between 30 and 40 % after 15 minutes and less than 60% after 60 minutes.

Arithmetic means MPA blood concentration vs. time profiles are shown in Figure 2 (0–48 h) and Figure 3 (0–6 h). Derived pharmacokinetic parameters for MPA and corresponding mean relative ratio (90% CI) are summarized in Table 1.

Comparisons of pharmacokinetic parameters between the 3 generics treatments showed apparent differences in peak MPA exposure (). C_{max} from generic 5 tablets was 22% lower on average than that from generic 1 tablets and the 90% CIs did not fulfill standard BE criteria (C_{max} ratio 0.78, 90% CI 0.64, 0.94). C_{max} from generic 8 tablets was also 16% lower on average compared with C_{max} from generic 1 tablets, while the 90% CIs did not fulfill standard BE criteria (C_{max} ratio 0.84, 90% CI 0.69, 1.02). Generic 8 and generic 5 showed comparable Cmax.

Total MPA exposure (AUC) was similar between all three generic tablet formulations () and the associated 90% CIs all lay within the range 80–125% for both AUC_{inf} and AUC_{last}. Variability in exposure parameters was similar between formulations (mean AUC_{inf} CV% 25–30%, mean C_{max} CV% 42–77%). Other pharmacokinetic parameters were also similar between formulations. Tmax lay between 0.67 hour to 1.33 hour.

No point to point relationship between the in vitro data and the PK parameters were found.

Level C was attempted between Cmax and AUC and percent of the dose dissolved in vitro at various times. The results are presented in Figure 4 for the % dissolved after 60 minutes (reflecting a mean Tmax) and Cmax. A strong relationship was found having a R^2 above 0.99. No relationship between the AUC versus in vitro dissolution was found.

Figure 1 Dissolution profiles of the 4 MMF tablets in acetate pH 4.5 buffer at 50 rpm.

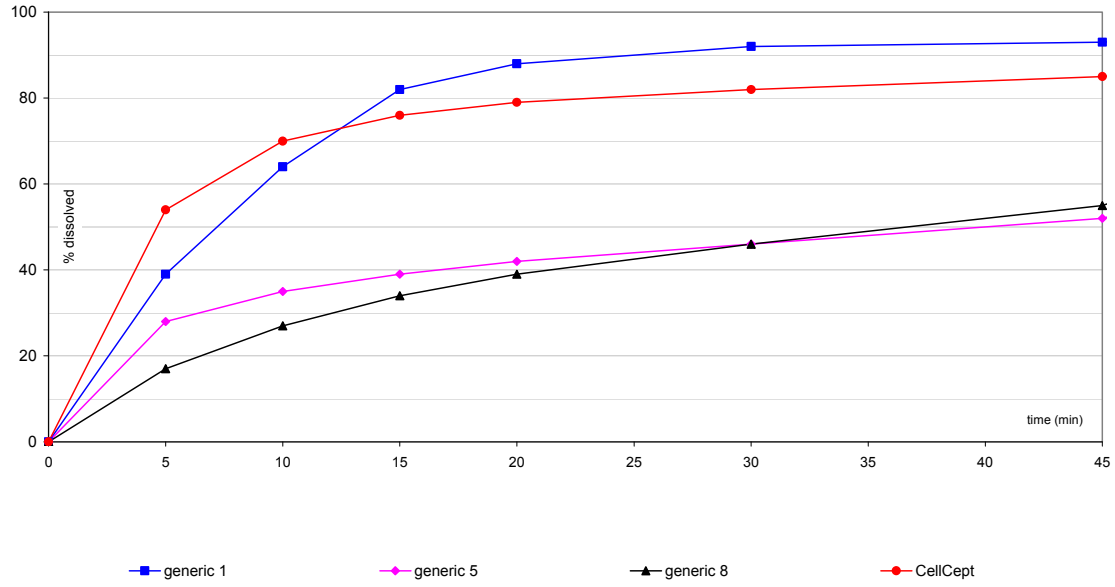

Figure 2 Mean MPA plasma concentration vs. time profiles (0-48h)

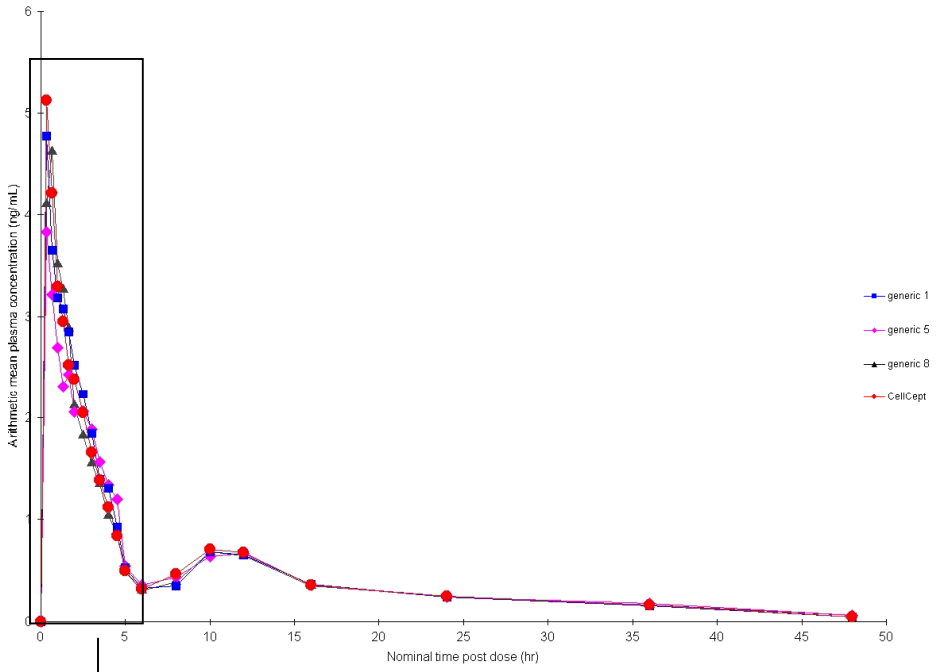

Figure 3 Mean MPA plasma concentration vs. time profiles (0-6h)

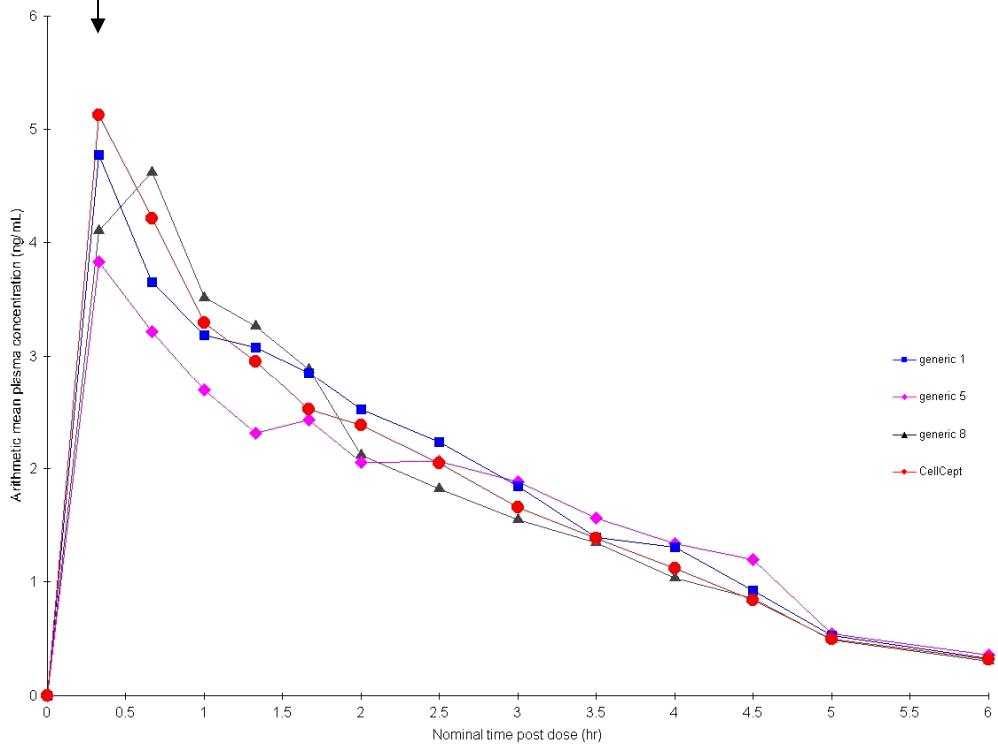

Figure 4 IVIVC level C, C_{max} versus % dissolved at 60 minutes

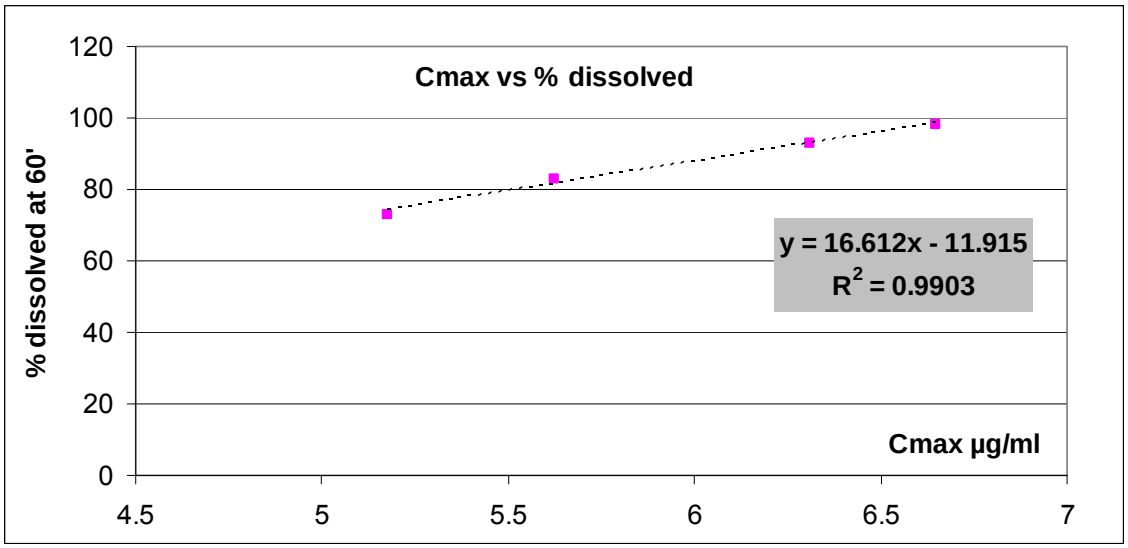

Table 1 Summary of MPA pharmacokinetic parameters

	Unadjusted means				Mean relative ratio (90% CI)		
	Generic 8 (N=31)	Generic 5 (N=32)	Generic 1 (N=32)	*CellCept* (N=32)*	Generic 8 vs. Generic 5	Generic 8 vs. Generic 1	Generic 5 vs. Generic 1
AUC_{last} (µg.h/mL)	20.72	20.40	21.10	*21.13*	1.01 (0.96, 1.07)	0.98 (0.93, 1.04)	0.97 (0.92, 1.02)
AUC_{inf} (µg/h/mL)	24.33	24.19	25.38	*26.5*	1.00 (0.94, 1.06)	0.96 (0.90, 1.02)	0.96 (0.90, 1.02)
C_{max} (µg/mL)	5.624	5.175	6.647	*6.312*	1.08 (0.89, 1.31)	**0.84 (0.69, 1.02)**	**0.78 (0.64, 0.94)**
T_{max} (h)	0.67	1.17	1.33	*0.67*	-	-	-

Geometric mean for AUClast, AUCinf, Cmax, t1/2

* for monitoring purpose only.

Discussion

The apparent differences in the peak exposures suggest there could be differences in the rate of absorption of MMF between the generic formulations. It is therefore possible that some generic tablets might not meet the accepted criteria for bioequivalence (ie, upper and lower 90% CIs within 0.80–1.25 for both Cmax and AUCinf) if tested against other generic tablets.

The apparent differences in clinical performance are consistent with observed differences in in vitro performance between generic 500 mg MMF tablet formulations. Dissolution testing in media simulating the environment in the proximal gastrointestinal tract showed marked variability in dissolution between generic formulations in some conditions. At pH 4.5 there was more than 60% difference in the amount dissolved after 30 minutes between the best and worst performing formulations.

Based on the IVIVC level C obtained with Cmax, (figure 4) the dissolution can serve as a good surrogate for the in vivo performance.

This strong correlation would enable researcher to better understand and appreciate the likely contributions that the formulations may produce in an *in vivo* study. For instance, if clear low performance in dissolution is observed for a new formulation, it may be reasonable to hypothesize that the *in vivo* peak concentration attainment could be lower.

Therefore, the rapidly dissolving properties of the MMF IR tablets in different pH can be considered as the CQA. Other examples concerning generics and IVIVC are reported in the literature (Bush 2009, Rouini 2008). This approach is in line with QbD strategy.

In our case the use of a simple and cost effective media allows additionally placing the dissolution as a more meaningful QC testing.

Conclusions

The dissolution tests in HCL used in routine and based on internal know-how of the company, is not enough to guaranty the quality of products which exhibiting similar quantity of API but different quality and/or quality of excipients. The tests performed had allowed setting up a simple technique that demonstrated differences between generic batches. Those differences were confirmed in vivo on one of the BE parameters. This approach which is a help for line extension and generic companies showed also that the simple dissolution test could discriminate between products. It shows as well the importance of having the right method early as possible to take the right decision. The example of Cellcept shows clearly the role of dissolution to identify the CQA, in this case the “rapidly dissolution” behavior.

Reference for supplement 2

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Conclusion experimental part 4

The central role of dissolution is well highlighted in this 4th part of the experiment. The identification of the rapidly dissolving behavior of the formulation at different pH is clearly the CQA. Therefore, differences in dissolution profiles can be useful predictors of clinical problems when drugs exhibit dissolution rate-limited absorption, particularly for BCS class II drugs.

As showed with this example of MMF formulations, the general need of having a strategy for brand protection for all drugs by developing discriminating dissolution methods is essential. Currently, generic applicants are required to utilize the compendial dissolution method when it exists. For non-USP products, generic applicants are frequently required to use an "OGD" (Office of Generic Drugs, FDA) method and associated specifications. Whether the method is a USP or OGD method, it may be not suited for the particular formulation. Generic products are often manufactured using excipients that are different than the brand counterpart. Manufacturing processes may also differ significantly. As such, dissolution methods and specifications that are appropriate for the brand product may not be suitable for the generic product.

Therefore, moving to a regulatory process that encourages quality by design principles, process understanding, dissolution methods and specifications that are based on product relevant characteristics is well acknowledged. This method helps then during the entire development and allows in this case by having an IVIVC in place to act as a strong surrogate for *in vivo* performance. Later for the generic industry, the quality by design approach creates the advantage of using "prior knowledge" that might include the following: (a) in vivo and in vitro performance of the reference product obtainable from the literature and/or experimental studies by the firm; (b) Biopharmaceutics, physico-chemical, formulation and dissolution characteristics of structurally related representatives of the same class of drugs. Many generic firms also have a large portfolio consisting of a wide range of product families. This prior knowledge should be leveraged as a resource to aid in the development, justification of tests and specifications for new products and ensure safety and similarity of generic for the patients.

This effort was made by Roche in order to have brand names of products not associated with therapeutic failure.

3.5. Summary of the experimental parts and discussion

In the various examples presented in the 4 experimental parts of this work we demonstrated that the dissolution could be applied on API and on formulations during the different development phases of a new medicine up to post launch. Factors likely to impact performance in vitro and ideally in vivo were investigated and identified using dissolution and IVIVC/R.
In the paper 1, we showed that the properties of the API (particle size distribution) is the key factor and that the in vivo performance is already well reflected thought the dissolution of the API. This example demonstrated how early dissolution could drive the parameter setting on API characteristics and on process optimization (e.g. choice of milling technique) leading, as mentioned in PAT and QbD, in a selection of more meaningful tests and specification which could insure a constant final quality of the product. This approach allows the key factors on API to be followed either through their direct monitoring or their impact on dissolution.

In the paper 2 and its supplements, the dissolution associated with IVIVC allows to identify the diffusion mechanism of an ER formulation as the main CQA. Support of theory and imaging allow a strong understanding of the release mechanism. Understanding dissolution and its mechanism should be integral to any method development. It helps for identification of CQA.
For both examples, the place of dissolution could easily be set up before or at the start of animal testing. With establishment of IVIVC between in vivo data obtained in animals and in vitro data, the dissolution supported the next step of formulation optimization. The selected formulations were found to be safe to be administered to man and the clinical study in human confirmed the pertinence of the choice. This approach allows to “cross validate” the animal species as surrogate for human performance and serve then as a strong derisking strategy.

In the paper 4, the rapidly dissolving properties of the IR tablets CellCept in different pH is a potential factor impacting the in vivo performance. It was proved that simple dissolution could also be implemented to insure the quality of finish products and generics. The dissolution tests used in routine and based on internal know-how of the company, is not enough to guaranty the quality of products which exhibiting similar quantity of API but different quality and/or quality of excipients. The tests performed had allowed setting up a simple technique that demonstrated differences between generic batches. Those differences were confirmed in vivo on one of the BE parameters (Cmax). This approach which is a help for line extension and generic companies showed also that the simple dissolution test could discriminate between products.

This effort was made by Roche in order to have brand names of products not associated with therapeutic failure.
All these examples highlight the strong advantage of having an IVIVC/R to support the development and post launch phases.

More complex methods, apparatus or non compendial instrument may be developed to aid in IVIVC/R discovery. In vitro this characteristic can be further challenged by changing the working condition as showed by using small volume vessel in paper 3 and its supplement. The small volumes dissolution exhibited the advantage to be more discriminant than the large volumes and to have a simple scaling factor. It's seems particularly interesting for BCS Class 1 and very rapidly disintegrating IR tablets where practically no alternative (apart disintegration) is available.

To predict in vivo behavior of BCS class II-IV drugs, simple and cost effective conventional media (HCl in paper 2 and acetate buffer in paper 4) or media with surfactant (HCL + 0.2% SDS in paper 1) were shown to be potential substitutes for the more complex, physiologically based Fa/FeSSIF. The use of simple buffer or biorelevant amounts of conventional surfactants in dissolution media will not only be economic during the various steps of drug development, but could also place the quality control dissolution tests into a more meaningful context.

All these applications highlight the central role of the dissolution.

There are several clear applications for dissolution during pharmaceutical development:

- Dissolution is a mandatory QC testing for DP. It allows to address batch to batch reproducibility, ageing. It can monitor CQA.
- Dissolution is used in early phase for screening of formulation performance in vitro.
- In later phases, dissolution serves as support for SUPAC, rules are followed even during development.
- In vitro dissolution results may be used as surrogate to predict the in vivo performance of drugs and formulations by either having an IVIVC or identifying CQA likely to impact in vivo performance.
- It supports waiver for bioequivalence (mainly BCS class1 and some class 3).

Other applications for dissolution testing are :

- the screening and characterization of salts, co crystals and polymorphic forms for appropriate selection during development.

- the screening for excipients (polymers, surfactants, etc.). These studies are useful to identify formulations with enhanced dissolution rates, crystallization/precipitation inhibitors or stabilizers.
- Dissolution testing has become an important tool in development for testing of powders. This can be applied for API and the galenical intermediates as pre blend and final blend to reflect the performance through the manufacturing process. This is well in line with the QbD and process understanding.

In summary, it is important to understand the mechanism that governs the release and solubilisation of the drug while developing a dissolution method; this is one of the primary goals during formulation development, along with the other goals of achieving bioavailability, uniformity, stability, and processability.

In practice the questions that arise at beginning of the development of a new dissolution method is “how should I start for a suitable method?” and “for what purpose?”

Even if we showed during this work some examples of a dissolution method addressing QC goals, QbD and biorelevance in once, in most of the cases it is acknowledged that QC dissolution tests (and purpose) may not reflect in-vivo physiological conditions. Furthermore, for drugs that exhibit low aqueous solubility (BCS II, BCS IV), surfactants are incorporated into the QC dissolution test in order to maintain sink condition during testing. The addition of surfactant to the dissolution media can reduce the discriminating capability of the dissolution test. What why the development of alternative methods in addition to classical QC is a current practice in the pharmaceutical industry. Several apparatus (USP1/2 and USP4) as well as several setups (pH change) are often applied in parallel during development.
It has to be kept in mind, that alternative dissolution methods may be highly sensitive to small perturbations in the drug product. This is particularly true for BCS category II and IV compounds. Therefore, variability inherent in these alternative dissolution methods may make them unsuitable for QC applications.
Decision trees are presented as synthesis of this work to foster the set-up of a dissolution method with regard to QbD and IVIVR/C for IR tablets (most frequently late phase formulation developed at Roche). Decision trees purely build on physicochemical properties such as logD, solubility, dissolution, solid state properties are rare (Lee 2003). More recently, the BCS classification scheme has been used for the selection and design of formulations (Ku 2008, 2010). The BCS (chapter 2.3.1) combines dose with solubility, permeability, and dissolution

and the derived parameters dose number (Do), dissolution number (Dn), and absorption number (An) may be used as more easily accessible decision criteria for building up simplified decision trees (Lennernäs 2005, Cook 2008). A decision tree for dissolution method development and for the selection of appropriate dissolution media for different categories of drugs has been proposed by Li et al. (Li 2005) based on the work from Dressman (Dressman 2000).The general issue with decision trees is that it tightly couples knowledge and the use of knowledge in individual decision paths and hence all possible decision paths and criteria have to be present in the tree. If information is missing, decisions can not be easily made. Therefore, the subsequent figure only intends to provide some basic insight and guidance to the reader on the rational behind dissolution method development to foster the set-up of individual method (Figure A).

In a first step, the compounds are classified according to their BCS properties. For Class 1 and 3 compounds, most simple yet reliable medium should be used, and no surfactant is needed. For compounds belonging to Class 2 and 4 SGF or SIF with suitable surfactant may be used. Fa/FeSSIF could be explored for API exhibiting sufficient solubility in such media or having a high logP value. PH-dependent solubility, dissolution of salt versus free form, and the distinction between weak acids and bases are then the key drivers when choosing the pH of a dissolution testing medium. A well USP3/4 apparatus can be an alternative to high amount of surfactant using USP 1-2 or by providing different hydrodynamics. At early stage (Phase 0-1) a worst case approach with regard to dose strength should be favored, since the final dose will not be known till end of phase 2 and the strength can vary. The method choice and rational should be justified so that it can be easily revisited as late phase changes occur.
In a second step the discriminatory power of the method is challenged by seeking relationship with API characteristics, granules particles size or tablets properties. The aim is to have the first inside of the CQA. A strong supportive database is a key for this step. Example of analytical methods to assess the quality of API, intermediate and DP is provided table A. These experiments should be designed on a case-by-case basis in consultation with the galenist, chemist and analytical specialist.

Figure A: Decision Tree: Dissolution development for IVIVC/R based on BCS approach.

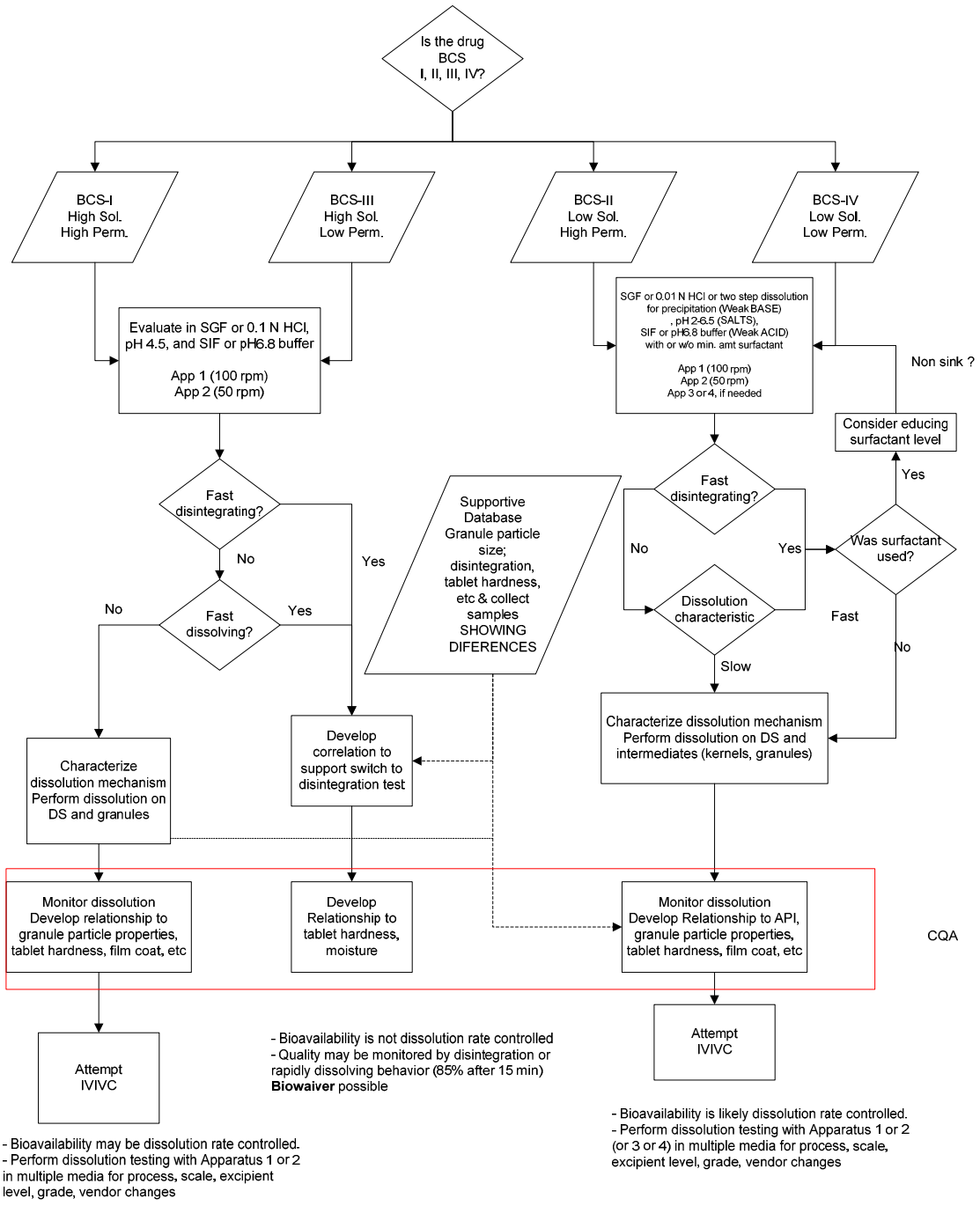


Table A: Example of methods that can support better process understanding for an IR tablet.

Intermediates	API	Pre beld	Final blend	Cores	Film-coated tablets	Main impact	
Bulk/tapped density	x	x	x			Granulation, flowability etc…	
Particle size distribution (PSD)	x		x			DR API	
Microscopy, Photos	x						
Shape factor	x						
SEM	x						
DSC/TG	x	x		(x)			
Specific surface area (BET)	x		(x)			DR API	
Dynamic vapor sorption	x					API moisture sorption	
XRPD	x	x		x	x	API amorph/crystall	
NIR, IR, Raman	x	x		x		API quality	
Wettability / sinkability	x					DR API	
Intrinsic dissolution	x		(x)			DR API, polymorph	P1
Apparent Dissolution USP 4	x	x	x	x	x	DR all factors	P1
Dissolution QC			(x)	x	x	DR all factors	P2
Dissolution alternative non sink,				x	x	DR	P4
Dissolution alternative small volume				x	x	DR	P3
Dissolution alternative 2 phases,				x	x	DR	
Chemical imaging (NIR, raman, µTC….)	x	x	x	x	x	Qualitative DP, process	P2
Stability (aggregation)	x					DR API	
Etc….							

In bracket corresponds to optional tests

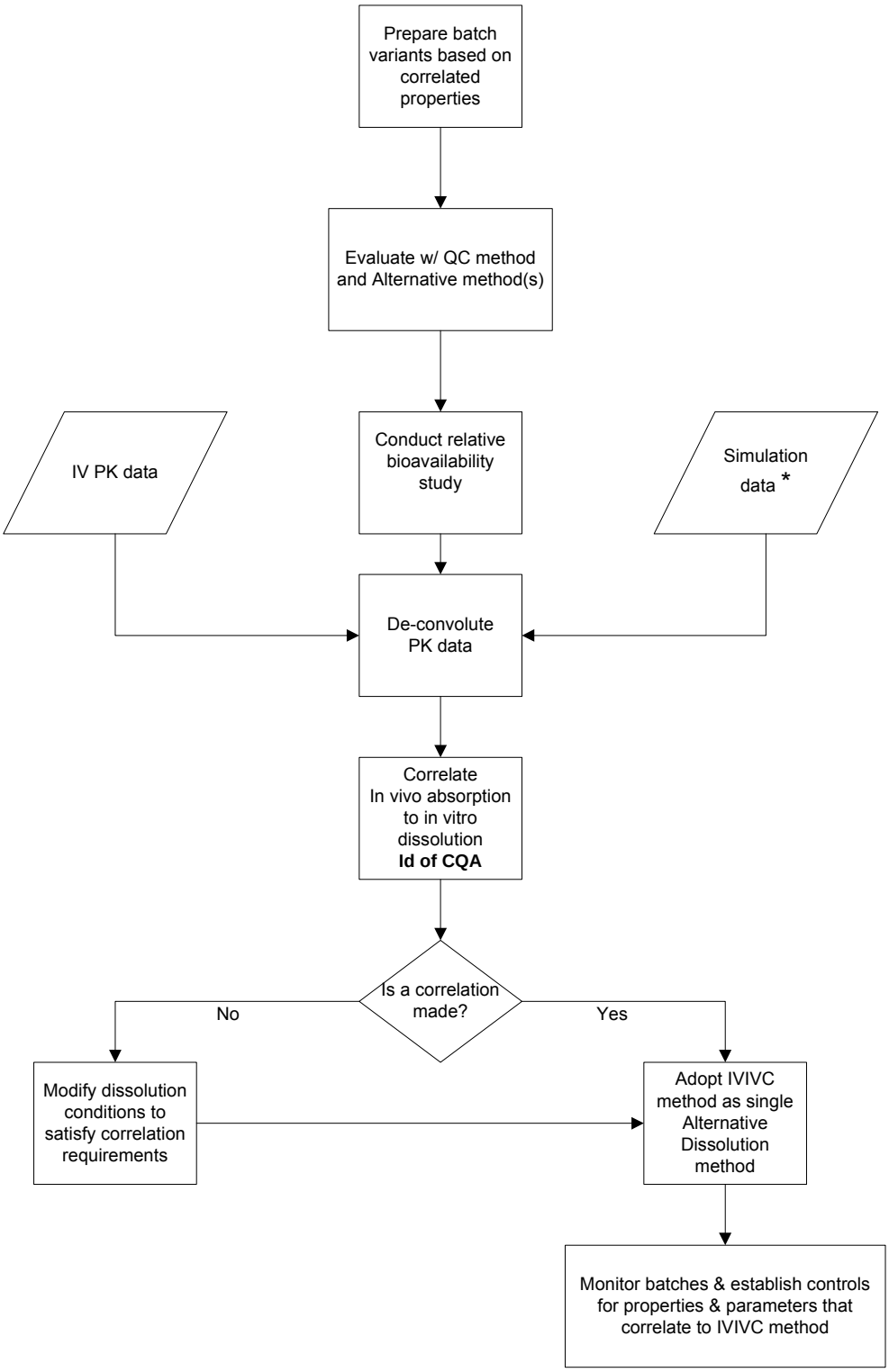
DR = Dissolution rate. DR All factors shows the dissolution as holistic testing.

Px indicates the main method used in the corresponding paper (Paper 1 to 4)

It is noted that due to the limitless range of conditions that may be employed in dissolution testing, a limitless range of dissolution results may be generated for the same product; results for which interpretation cannot readily be drawn with respect to inferences on quality. In an effort to simplify the analysis of data generated from multiple methods, the principle of risk analysis is applied to alternative dissolution testing. Dissolution testing may discriminate for variations in the drug substance, excipients, manufacturing process, storage conditions. The main risk is that any change from one or more of these parameters may result in a change in in-vivo drug release, thereby posing a safety risk to the patient. Therefore, correlation of the

dissolution method(s) to in vivo drug release is a necessary component of dissolution test development (i.e. IVIVC). A general approach to establishing an IVIVC is provided in Figure B.

Figure B: Decision Tree : Dissolution development for IVIVC/R attempt



* see Chapter 2.4.3 for detailed approach.

Finally, it should be emphasized that an understanding of the design of the pharmaceutical dosage form is critical to facilitate the design of an appropriate test, and to avoid artifacts created by a poorly designed test. Considerations of the dosage form design may include: an immediate release versus modified release product; eroding versus disintegrating dosage form, expected behavior of excipients used in the formulation under agitated, non-agitated, or minimally agitated condition

Reference for Summary and Discussion

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4. Conclusion

The value of in vitro dissolution testing as a quality control tool is demonstrated by its long history of regulatory acceptance (it is included in Pharmacopeia since 1970). In the present work we tried to demonstrate that the dissolution tests exhibit a broader range of possibilities starting from preclinical research up to post launch surveillance of product quality. In early phases focus is on screening where it is important to select the right API, the right formulation and to identify the critical manufacturing parameter whereas for release consistent batch manufacturing and stability testing are the main focus. During development, dissolution testing can be a sensitive and a reliable predictor of bioavailability and tool for biowaiver.

As exemplified in this thesis, simple and cost effective dissolution methods were shown to be potential surrogate for in vivo performance and serve as well for strong QC method. Dissolution was used as a surrogate marker or a supra indicator of all processes which are involved in the quality of the API or the formulation as well as in manufacturing process. With this regard the dissolution acts as strong tools for QbD. In all these examples the central role of the dissolution during development up to post launch phases was perfectly shown.

Although success has been achieved as showed during these investigations, further effort and more expertise need to be invested in the development of dissolution methods. Tools like DoE or simulation as well as the BCS/BDDCS and new regulatory QbD directives will lead to more innovative and science-based approaches in order to ensure the dissolution consistency of the oral dosage forms. All those examples demonstrated that more investment on dissolution already in early stage, and in particular for API, is valuable, it is easy to set up, fast and cheap compared to full development or even to a BE study and allow taking the right decision earlier with minimum risks insuring a global safety and efficacy of the products. Pharmaceutical industries should even further optimize their organizations in this direction, for instance by centralization of the dissolution activities and by putting emphasis on cross functional effort, having pharmaceutical scientists, chemist and analytical specialists working together.

There is an expectation that the changing paradigm in dissolution method development will lead to an increased of IVIVC/R attempts. With the challenges associated with IVIVC, especially for IR dosage forms, IVIVR should be increasingly leveraged to support QbD and design-space development. Even if these investigations are not fully successful in term of bio relevance or prognostic, the efforts invested will gain enough information to be more confident on all critical aspects that may impact the quality of the drug product (CQA, manufacturing). In summary dissolution is a strong tool to fasten development and increase quality when properly associated with IVIVC/R and QbD.

The efforts to improve the dissolution testing will allow for increasingly rational drug development, sound specification setting and strong regulatory and derisking tools. It is valuable if academia, industry and regulatory agencies could put more emphasis on devising predictive dissolution testing and if pharmaceutical scientists could work together further in this pursuit.

Résumé

La dissolution est considérée comme une des méthodes clés durant le développement d'une forme pharmaceutique et pour le suivi de la qualité après mise sur le marché. En phase de développement précoce l'étude de la vitesse de dissolution est utilisée dans la sélection des formulations avant les études toxicologiques et les premiers tests sur l'homme. En phases de développement avancées la dissolution est réalisée principalement pour comparer de nouveaux prototypes, optimiser le procédé de fabrication, s'assurer la reproductibilité de lot à lot et évaluer le suivie de stabilité. Bien que la dissolution *in vitro* soit une méthode précisément décrite et largement utilisée dans l'industrie pharmaceutique, plusieurs défis existent encore dans ce domaine d'application. En particulier en ce qui concerne l'identification et de la compréhension des différents paramètres critiques qui contrôlent la libération du principe actif (PA) pure et à partir de sa forme pharmaceutique. Avec l'établissement de corrélations *in vitro/in vivo* (IVIVC) la dissolution se place alors comme un indicateur sensible et fiable des performances *in vivo*.

Ce travail se concentre sur l'utilisation optimum des méthodes de dissolution existantes et explore quelques alternatives simples pour poser les fondations des approches de « Quality by Design » (QbD) et des corrélations *in vitro/in vivo* (IVIVC). La dissolution appliquée au PA et à différentes formes pharmaceutiques (libération immédiate et retardée) et ceci à différentes phases du développement ainsi que pour les génériques a été explorée. Les résultats obtenus ont permis la sélection de méthodes de dissolution de control qualité simples et peu couteuses qui idéalement peuvent aussi servir de test de substitution pour la prédiction de la performance *in vivo*. Les perspectives futures et le rôle central de la dissolution sont présentés et discutés.

Mots-clés: dissolution *in vitro*; correlation in *vitro/in vivo* (IVIVC).; relation *vitro/in vivo* (IVIVR); Biopharmaceutical Classification System (BCS); Quality by Design (QbD); générique.

Abstract

Dissolution has emerged as a key method during development of medicines and for quality control of marketed products. At the early stage of development, dissolution guides the selection of toxicology and first test in man formulations. At later stages of development, dissolution tests are performed to compare prototype formulations, the robustness of the manufacturing process, to indicate stability and to assure safe release and reproducibility of the products to the market. However despite they wide use in pharmaceutical development, several challenges still exist. In particular, there is a lack of thorough identification and understanding of the critical quality attributes that control dissolution of Active Pharmaceutical Ingredient and Drug Product. Dissolution exhibits clearly a higher predictability if it can be extrapolated directly to in vivo behavior.

The present work focuses on the optimization of the existing and alternative dissolution techniques to lay a foundation for Quality by Design (QbD) principles, *In Vitro/In Vivo* Correlation (IVIVC) and *In Vitro/In Vivo* Relationship (IVIVR). The dissolution applied on API and on different formulations types (Immediate release and extended release form) during the different development phases as well as for generic has been explored. Simple and cost effective dissolution methods were shown to be potential surrogate for *in vivo* performance and serve as well for strong quality control method. The future perspectives and central role of dissolution testing are presented and discussed.

Key words: *In vitro* dissolution; *In Vitro/In Vivo* Correlation (IVIVC); *In Vitro/In Vivo* Relationship (IVIVR); Biopharmaceutical Classification System (BCS); Quality by Design (QbD); generic.