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Workshop Report: USP Workshop on Advancements in In Vitro Performance Testing of Drug Products

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ABSTRACT

In December 2019, The United States Pharmacopeia (USP) organized a 2-day workshop to explore new approaches to assess in vitro performance of drug products. Experts from around the globe presented processes, techniques, systems that can be used to evaluate and model in vitro performance of different pharmaceutical dosage forms. The following is a summary of most of the presentations and the highlights of the discussions that ensued.

Disclaimer: This article reflects the views of the authors and should not be construed as representing the views or policies of the United States Food and Drug Administration.

KEYWORDS: in vitro performance, modeling, dissolution, food effect, biorelevant, nanomaterials, real-time release test.

INTRODUCTION

Because of their close association with performance tests for pharmaceutical dosage forms, in vitro dissolution and/or disintegration are required to better control the critical quality attributes (CQAs) of the product and to understand their performance in vivo. Additional, in vitro procedures and modeling tools can used to facilitate formulation development and optimization, post-approval changes, pre-assessment of food effect, etc. A particular challenge is to develop and implement a dissolution test in a continuous manufacturing environment.

To discuss the challenges in developing in vitro performance tests for dosage forms other than tablets and capsules, and the tools and models that can be used to develop those tests, the United States Pharmacopeia (USP) sponsored a 2-day workshop (December 11–12, 2019) convening international experts to share their experiences in assessing in vitro performance tests for pharmaceutical dosage forms. This report contains

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highlights of the workshop presentations and some of the questions and answer sessions from the panel discussion sessions.

Common abbreviations used in this workshop report include:

- 3D: Three-dimensional
- API: active pharmaceutical ingredient
- AUC: area under the curve
- BCS: Biopharmaceutics Classification System
- BE: bioequivalence
- CQA: critical quality attribute
- EP: European Pharmacopeia
- GI: Gastrointestinal
- IVIVC: in vivo-in vitro correlation
- IVPT: in vitro permeation test
- IVRT: in vitro release test
- JP: Japanese Pharmacopoeia
- ODT: orally disintegrating tablets
- OIDP: orally inhaled drug product
- PAT: process analytical technology
- PBBM: physiologically based biopharmaceutics model
- PBS: phosphate-buffered saline
- PK: pharmacokinetic
- QC: quality control
- RTRT: real-time release test
- SSF: simulated saliva fluid
- TDS: transdermal delivery system
- US FDA: United States Food and Drug Administration
- USP: United States Pharmacopeia

FIRST-PRINCIPLES APPROACHES AND SURROGATE TESTING FOR PREDICTING IN VITRO DISSOLUTION

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Modeling and predicting the in vitro dissolution of dosage forms can be a powerful tool during drug development as well as for commercial QC purposes. In general, the dissolution behavior can be predicted based on the physicochemical properties of the API and the dosage form via fundamental first-principles approaches, empirical modeling, or a hybrid of these two. Although dissolution models based on first principles are often most useful during early formulation development, empirically models based on PAT methods, which can correlate to dissolution, are more commonly employed in later development once a final formulation has been defined. Building these models can significantly support and enhance drug development because these models require close examination factors that impact dissolution including general drug properties as well as changes in formulation and process. With this understanding, more robust control strategies and product quality can be developed. Further, dissolution is an inherently slow test, which is hard to implement on the manufacturing floor. This makes it not suitable as an RTRT. With increased emphasis of continuous manufacturing in pharmaceutical applications, development of dissolution models and alternative real-time performance (surrogate) tests that can replace dissolution testing helps enable the possibility for RTRT, which is particularly challenging for low solubility compounds.

To develop mechanistic first-principles dissolution models for solid oral dosage forms, each step of the overall dissolution process (i.e., film coat removal → tablet core disintegration into granules → granule disintegration → API] dissolution) is commonly examined separately and explained based on physicochemical properties. The API dissolution step especially is very well understood and can be modeled based on the Noyes-Whitney or related equations. Models based on first-principles dissolution are often used during drug formulation optimization and for in vivo physiologically based pharmacokinetic (PBPK) modeling. Empirical dissolution models typically often make use of fishbone diagrams as a useful visual tool to illustrate which material attributes and process parameters can impact the overall dissolution behavior. Based on risk assessment, the most influential parameters are varied through targeted experimentation and the resulting dissolution behavior is correlated with the factors in order to build empirical dissolution models. Often, additional test results from spectroscopic methods such as near-infrared (NIR) measurements are incorporated as factors to predict the dissolution response.

Dissolution modeling is not only restricted to predicting dissolution for immediate release oral dosage forms. As example, for osmotic pump tablets, terahertz spectroscopy has been successfully used to determine the thickness of the semipermeable membrane, which dictates the dissolution behavior of osmotic pump tablets. This spectroscopic technique can predict tablet-dissolution

performance on a much faster timescale than traditional dissolution testing. The time savings are even more pronounced when long-acting, implantable formulations are considered. Irizarry et al. demonstrated that analysis of x-ray computed tomography (XRCT) images from binary drug/polymer implant systems is capable to accurately predict the dissolution behavior of such dosage forms at a fraction of the time that real-time dissolution would deliver, which might take up to several years.

THREE-DIMENSIONAL (3D) PRINTING FOR FAST PROTOTYPING OF PHARMACEUTICAL DISSOLUTION TESTING EQUIPMENT FOR NONSTANDARD APPLICATIONS

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3D printing technology is rapidly spreading into the daily life, changing philosophy of design, manufacturing, and logistics in various areas of human activity. In the pharmaceutical sciences, 3D printing is extensively tested as novel method of drug product manufacturing, but the possibility of rapid prototyping analytical equipment or its parts is rarely discussed. 3D printing technologies allow for easy manufacture of various setups for drug dissolution testing, for example:

1. Dedicated equipment for rare or nonstandard drug delivery systems, i.e., buccal mucoadhesive formulations, vaginal formulations, controlled release drug delivery systems, gastroretentive floating formulations, wound dressings, implants, etc.
2. Equipment for dissolution studies combined with additional analytical techniques, i.e., magnetic resonance imaging (MRI), computed microtomography (microCT) various microscopy techniques, etc.
3. Equipment for dissolution testing in biorelevant conditions (chambers with different size geometry, with moving parts, etc.).

Among various 3D printing techniques, the most feasible for the preparation of dissolution equipment are stereolithography (SLA), fused-deposition modeling (FDM), and selective laser sintering (SLS).

Figure 1 presents an example of the FDM modeling of MRI-compatible dissolution insert to the flow through cell (USP apparatus 4) developed for mucoadhesive buccal tablets.

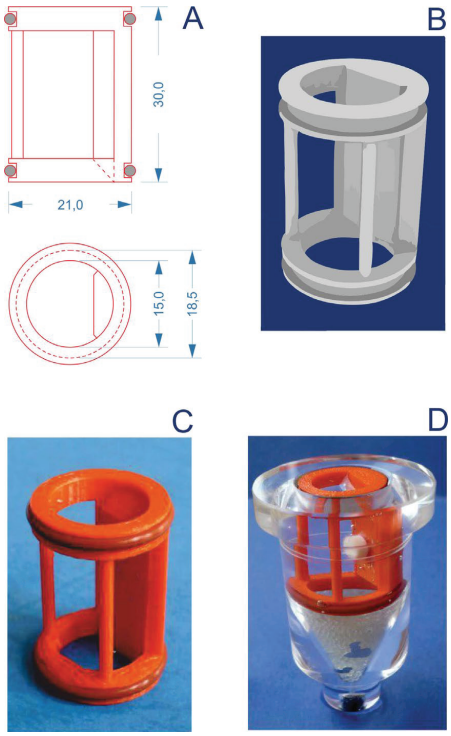

Figure 1. The example of the FDM modeling of MRI-compatible dissolution insert to the flow through cell dedicated for mucoadhesive buccal tablets: (A) technical drawing of the insert, (B) 3D model of the insert, (C) printed insert, and (D) insert with the tablet fitted inside the flow-through cell (2). FDM: fused-deposition modeling; MRI: magnetic resonance imaging; 3D: three dimensional.

Rapid prototyping techniques were found to be a fast, inexpensive way to develop a dedicated solution for dissolution testing. The advent of novel approach to equipment manufacturing, brings opportunities for its design and development, expanding the application possibilities, matching the equipment to specific needs creating libraries of files for printing and sharing them. It also raises issues regarding the standardization and qualification of designed and printed items of equipment.

The development of the 3D printed setups for dissolution studies is a part of the project (NOMAD-L), which is dedicated for development of innovative testing methodology for drug products under development granted by The National Center of Research and Development (POIR.04.01.04-00-0142/17-00).

DISSOLUTION MODELING FOR REAL-TIME RELEASE TESTING (RTRT)

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Continuous manufacturing and RTRT are at the forefront

of pharmaceutical industries' innovative endeavors and are supported by health authorities. The combination of continuous manufacturing and inline PAT monitoring enables building quality-by-design into the complete product lifecycle, with the ultimate goal of getting high quality medicines to market in a more efficient and cost-effective way. RTRT is achieved through the analysis of up-stream, in-process materials to assess the end product's CQAs against the release specifications. Out of all the CQAs for RTRT, modeling for dissolution may be the most challenging, as tablet dissolution is often influenced by many material attributes and process parameters.

In this presentation, dissolution modeling of a fixed-dose combination (FDC) tablet with two APIs was presented. Correspondingly, two RTRT dissolution models have been developed. A comprehensive understanding of the drug product formulation and manufacture process was essential to establish the RTRT model.

For each input factor that could potentially influence tablet dissolution, PATs have been implemented, with measurements taken at different stages of the process. An RTRT dissolution model was then developed via a step-wise approach based on modified Noyes-Whitney equation. Model development is just the first step of the full RTRT model lifecycle, which also includes model assessment, model validation, model transfer (if needed), and model maintenance. The health of a model is assessed through its life cycle. Model update can be triggered in several ways, including, but not limited to, routine parallel testing, process or material changes, etc., and supplemental validation will be executed once model update is made.

In summary, development and maintenance of RTRT models are continuous efforts. Continuous manufacture and RTRT are innovative, data-rich approaches that lead to high-quality products.

BIORELEVANT IN VITRO GASTROINTESTINAL MODEL (tTIM-1) FOR FOOD-EFFECT PREDICTION

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In vitro dissolution testing is frequently used in pharmaceutical formulation development to predict in vivo drug solubility/dissolution. Standard in vitro methods are limited in their ability to simulate the dynamic aspects of in vivo dissolution of a dosage form during transit through a rapidly changing and complex GI environment, especially when effect of food is involved. Food intake can have significant effect on GI performance of oral drug product and the change in pharmacokinetics induced by food could have impact on safety or efficacy for narrow therapeutic-index drugs. Understanding the risk of food effect for a new drug in early formulation development can help in mitigating the food effect through formulation technologies and in designing clinical trials.

In this presentation, an advanced in vitro, GI model, tiny TIM-1 (tTIM-1), was evaluated for its feasibility to predict in vivo bioperformance of solid oral formulations under fast and fed conditions. The in vitro TNO (Netherlands Organization for Applied Scientific Research) GI model (tTIM-1) is a computer-controlled, in vitro GI system designed to perform dynamic dissolution testing in presence of physiologically relevant parameters such as media, volumes, hydrodynamics, etc. The tTIM-1 system is a simplified version of TIM-1, which consists of a gastric compartment and a single small-intestinal compartment. It eliminates unnecessary complexity associated with the original TIM-1. Two low-solubility compounds (a weak base and a weak acid) in tablet dosage form at various doses were tested. The tTIM-1 was used with standard protocols for fasted and fed conditions. For the fasted state, the tablets were given with 240 mL of water in the stomach compartment filled with 10 mL of gastric fluid. For the fed state, tablets were given with a homogenized mixture of a standard high fat meal as recommended for clinical studies by the US FDA. The average conditions in the upper GI tract of healthy adult humans (gastric emptying and housekeeper waves, pH gradient, digestive enzymes, etc.) were simulated by the tTIM-1 system. The amount of the dissolved drug in the intestinal chamber, collected after filtration through a hollow fiber semipermeable membrane per time period, was considered as the fraction available for absorption from the upper GI tract, i.e., the bioaccessible amount within a given time period . The bioaccessibility profiles of the drug were compared with human data.

In the first example, clinical data show that both AUC and C_{max} plateaued out with increasing dose in fasted condition and positive food effect was observed in fed condition and this food effect is more significant at higher doses. The bioaccessible amounts generated with the tTIM-1 at various doses show a strong correlation with in vivo AUC for both fasted and fed conditions. The food effect ratios (fed/fasted) predicted by tTIM-1 are less than those in vivo especially at higher doses. This compound is known to be a substrate of a P-glycoprotein (P-gp)

efflux pump. The higher human food effect ratios suggest that other physiological mechanisms such as food-induced competition or inhibition of P-gp transporters may play a role in addition to solubilization by food. For the tTIM-1 system, sample filtration rate (a surrogate for permeability) could have great impact on measured bioaccessibility. For this compound, a higher sample filtration rate instead of a standard filtration rate of 3 mL/min may further improve food effect prediction accuracy. In the second example, in vitro solubility data show a lower solubility in simulated fed state media than in fasted state condition, suggesting a potential negative food effect. The tTIM-1 data show a higher bioaccessible amount and a delayed T_{max} in fed condition that are consistent with the human data – a positive food effect. In this case, solubility data in simulated fasted and fed media do not reflect in vivo observed food effect. One possible explanation for the observed positive food effect (both in vivo and tTIM-1) may be due to the food digestion process where change in lipid/digestion product composition during digestion favors drug solubilization resulting in higher drug concentration in the intestine lumen available for absorption. A very good correlation between in vitro tTIM-1 bioaccessible amount and human in vivo AUC at two dose levels for both fasted and fed conditions was obtained for this compound.

In summary, the tTIM-1 system can be used as a useful in vitro tool in early formulation development to assess bioperformance risks for food effect in humans, which is often difficult to simulate using standard in vitro methods.

THE BIORELEVANT GASTROINTESTINAL TRANSFER (BIOGIT) SYSTEM FOR ASSESSING THE IMPACT OF DOSE AND FORMULATION ON EARLY EXPOSURE AFTER ORAL ADMINISTRATION

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The biorelevant gastrointestinal transfer (BioGIT) system is an open, in vitro setup for assessing apparent drug concentrations and percentage of solid fraction in upper small intestine, after co-administration of a solution, suspension, disintegrating, or dispersing dosage form with a glass of water to fasted adults. In case of solution formulation, the percent precipitated in the upper small intestine is also assessed. Using part of the sample collected for measuring apparent drug concentration, apparent equilibrium solubility can further be measured and, therefore, apparent supersaturation in upper small intestine can also be assessed.

In vitro conditions are based on volume of duodenal contents, and drug input/output duodenal rates estimated, after modelling luminal data of highly permeable drugs. The conditions in the duodenal compartment consider both the transport of a highly permeable drug via the epithelium of upper small intestine and the transit along the lumen of upper small intestine. Unlike an initial attempt, BioGIT methodology complies with the continuous GI transfer process whereas the in vitro setup comprises commercially available equipment.

In pharmaceutical research and development (R&D), the BioGIT methodology has been shown to be useful in

- Understanding the impact of GI transfer on drug concentrations in the upper intestinal lumen, after oral administration of disintegrating solid dose units, suspensions, or solutions to fasted adults with a glass of water.
- Assessing the precipitated dose fraction in the upper GI intestinal lumen after oral administration of disintegrating solid-dose units containing the drug in solution or of a drug solution to fasted adults with a glass of water.
- Assessing the impact of formulation and dose on early exposure after oral administration of disintegrating solid dose units, suspensions, or solutions to fasted adults with a glass of water.
- Understanding the performance of drug complexes with ion exchange resins in the upper GI lumen, after oral administration to fasted adults with a glass of water.

BioGIT could also be useful in the regulatory setting in

- Providing supporting information on the impact of dose and formulation on early exposure after oral administration of disintegrating dose units, suspensions, or solutions in the fasted state, and
- For informing PBBMs on precipitation kinetics in the upper small intestine.

DISSOLUTION FOR PRODUCTS APPLIED TO THE ORAL CAVITY

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Products applied to the oral cavity represent a heterogeneous group of dosage forms that are usually placed in the oral cavity without coadministration of fluid. Reasons for their application include, but are not restricted to, proper administration of drugs in patients that face difficulty in swallowing (e.g., pediatric or geriatric patients), increased patient compliance, the provision of quick onset of action, avoidance of first-pass metabolism or degradation in the GI tract, or attainment of local action in the oral cavity or throat.

The dosage forms can be placed at different sites in the oral cavity, e.g., on the tongue, under the tongue, or in the buccal pouch. They shall either disintegrate, disperse, or dissolve in the oral cavity, release drug in the oral cavity or GI tract, or show local or systemic action (absorption via the oral mucosa and/or the GI tract) and can be immediate-, delayed-, or extended-release formulations. The classification of the different types of dosage forms differs among international pharmacopoeia such as the USP, EP, and JP.

Official Dissolution Test Methods

The USP contains a few individual dissolution methods for lozenges, ODTs, chewable, and sublingual tablets. All official methods prescribe the use of the paddle (apparatus 2) or the basket (apparatus 1) apparatus and 500–1000 mL of water or another aqueous medium. These test conditions are far from simulating conditions in the oral cavity, which indicates the QC character of these methods. The USP <1004> "Mucosal Drug Products – Performance Test" also refers to the potential use of a miniaturized paddle or basket setup for assessing in vitro drug release of sublingual and buccal tablets and films, the use of the paddle over disk (apparatus 5) apparatus for films, and the reciprocating cylinder (apparatus 3) apparatus for lozenges. Finally, it indicates that for some experiments, a physiological medium might be required.

The EP does not contain individual or general methods for products applied to the oral cavity. Chapter <2.9.25> describes a general dissolution test procedure for medicated chewing gums and two different chewing apparatus that can be applied for in vitro dissolution testing of these dosage forms.

The JP does not contain any general monograph on how to assess in vitro dissolution of products applied to the oral cavity. The paddle method, a fluid volume of 900 mL, and the use of aqueous dissolution media with and without surfactants added are prescribed in individual monographs for ODTs and a chewable tablet formulation.

The Dissolution Methods Database of the US FDA provides information on dissolution methods for drug products that do not have a dissolution test method in the USP and contains the largest number of recommended dissolution methods that is currently available for products applied to the oral cavity. Interestingly, for a chewing gum product, it refers to the chewing apparatus described in the EP and to a mini-basket apparatus for assessing drug release from buccal films. All other methods are similar to those described in USP and JP. From screening all official methods available today, it is clear that they were developed for QC, but are unlikely to be biopredictive.

Anatomy and Physiology of the Oral Cavity

When the aim is to better predict the in vivo performance of drug products applied to the oral cavity, it is important to consider the specific anatomy and physiology at the site of administration. Whereas the buccal cavity is confined to the inner cheek area, the oral cavity is located between the dental arches, partly filled by the tongue and separated from the nasal cavity by the palate. The oral cavity is lined by relative smooth mucous membranes containing salivary glands that secrete about 1–2 L of saliva per day. Different saliva secretion rates can be observed. Since saliva is swallowed at regular intervals, the resting volumes in the oral cavity are quite small. What is known under the general term "saliva" is a mixture of fluids secreted by the different types of salivary glands and thus of variable composition. Overall, saliva is a slightly viscous, hypotonic fluid with a pH in the range of 6.2 (high secretion rates) to 7.4 (low secretion rates), which is primarily composed of water (~ 99%), mucus, proteins, mineral salts (e.g., Na^+, K^+, Cl^-, HCO_3^-, Mg^{2+}, PO_4^{2-}), and amylase.

Novel Dissolution Test Methods

The official methods currently available for products applied to the oral cavity were developed for QC, i.e., to discriminate, but not to be predictive for, in vivo drug release. Biopredictive, in vitro test methods for these products will need to address the parameters that are critical to drug dissolution and absorption in the oral cavity and/or human GI tract, i.e., the residence times, pH, fluid volume, composition and exchange rates, and mechanical impacts such as tongue agitation and chewing.

In the recent past, various novel dissolution methods have been proposed. The majority were developed for orodispersible formulations such as particles and taste-masked particles, sublingual tablets, and films. Methods range from modifying the basket (apparatus 1), compendial paddle (apparatus 2), or flow-through cell (apparatus 4) setups by inserting special 3D-printed dosage form holders for oral films, through simulating the small fluid volume and flow rates in the oral cavity in a self-made, mini-column apparatus for dissolution screening of taste-masked particles, to more advanced methods for screening dissolution of (taste-masked) particles in the oral cavity and after swallowing. Where in the modified compendial setups for oral films high fluid volumes and compendial dissolution media were applied, the cited test methods developed for orodispersible particles aimed to address physiological fluid volumes and saliva composition. For simulating composition and properties of human saliva, various types of simulated saliva fluids (SSFs) have been published in the literature. Most of them had originally not been developed for in vitro dissolution testing of pharmaceutical dosage forms. Although, for the purpose of biopredictive, in vitro dissolution testing they might be further refined in the future, the use of one of these SSFs represents a good starting point for simulating conditions in the oral cavity. When testing orodispersible and orally disintegrating formulations, it should be noted that oral dispersion or disintegration does not indicate that the drug will dissolve in the oral cavity. Thus, a dissolution test that solely focuses on the simulation of conditions in the oral cavity might not be effective. As such, there is a need to equally address other GI segments such as the stomach and the small intestine, particularly for formulations with delayed or extended release, and also for taste-masked formulations that will disperse in the oral cavity, but be swallowed before drug release initiates. By contrast, for formulations that rapidly disintegrate and concurrently dissolve in the oral cavity, a properly designed disintegration test might be used as a product performance test.

To date, virtually no method has been described for assessing drug release from buccal tablets and buccal films. Because these formulations are administered for systemic drug administration and will be attached to the buccal tissue and immersed in the small fluid volume available in the buccal cavity, a vertical diffusion cell or an immersion cell setup combined with biorelevant fluids might be a good starting point for a successful method development.

For lozenges, the USP recommends the use of apparatus 1–3. With these setups, it is hardly possible to simulate the concerted action of tongue and hard palate as well as saliva secretion and swallowing. Tietz et al. proposed a new, in vitro model that enables the assessment of local drug availability and BE of locally acting lozenges. The novel, in vitro setup addresses a number of parameters relevant to drug release in the oral cavity such as tongue agitation, which results in pressure and shear stress acting on a lozenge during sucking, and the small saliva volume available in the oral cavity, which is refreshed by stimulated saliva flow, and swallowing in regular intervals. Using the novel in vitro model and SSF as the dissolution medium, they were able to obtain an IVIVC for drug release and mass loss of the lozenges, which was an important step forward in establishing biopredictive test methods for lozenges.

Two chewing apparatus are described in the EP. Surprisingly, although these apparatus have been official for several years and represent a valuable tool, when the aim is to address the impact of small saliva volumes and chewing actions on drug release from chewable tablets and medicated chewing gums, none of the apparatus have been mentioned in the USP. It is likely that this might happen soon.

Summary

In summary, both the classification of the different dosage forms applied to the oral cavity and the in vitro methodology applied in QC differ between pharmacopoeia. For many dosage forms, in vitro dissolution methods for QC have not been described. Furthermore, many of the official methods described to-date are unlikely to be applicable for predicting the in vivo performance of the different types of products applied to the oral cavity. Recently, various novel, in vitro dissolution methods have been proposed in the literature, but for many of the proposed methods, the intended use has not been clearly stated. Several of the newer methods present a mix of compendial and biorelevant test parameters, but, aside from a few exceptions, real biopredictive methods are still lacking.

A proper method development for both QC, and prediction of the in vivo performance of products applied to the oral cavity, requires detailed knowledge of the anatomy and physiology of the oral cavity and the upper GI tract. For developing appropriate in vitro tests, there is also a need to know all essential formulation details and to further specify the target in vivo release profiles

of the dosage forms to be tested so that both information on physiology and formulation characteristics can be implemented in the development and fine-tuning of discriminating and biopredictive, in vitro dissolution methods. Finally, in vitro dissolution method design would greatly benefit from a global harmonization of the nomenclature of products applied to the oral cavity.

UTILIZING PREDICTIVE, IN VITRO METHODOLOGIES TO GUIDE SUCCESSFUL DEVELOPMENT OF GASTRORETENTIVE DRUG DELIVERY SYSTEMS

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Gastroretentive (GR) formulations can be very useful to provide therapeutic efficacy of drugs that have a narrow absorption window, are unstable at alkaline pH, are soluble in acidic conditions, and are active locally in the stomach. The physiological state of the stomach provides significant challenges to develop GR formulations. Approved products using gastrointestinal technologies include those that are expandable, mucoadhesive, and magnetic, as well as ion-exchange resins, and low- and high-density systems. The motility of the stomach could break down non-resilient dosage forms and high-intensity "housekeeper waves" could clear indigestible material from the stomach. Hence, understanding formulation robustness and swelling capability to avoid premature gastric emptying, is important. Given the lack of good preclinical animal models for GR systems, it is imperative to rely on predictive, in vitro analytical tools for optimal PK performance.

In a case study to predict the GR properties of the dosage forms, various in vitro, analytical methodologies such as disintegration, USP apparatus 1 using a bolus basket, and apparatus 3 were found to be suitable to investigate drug release mechanisms, swelling/erosion profiles, and robustness of the GR formulations under fasted/fed state. The examined GR formulations were determined to swell to at least twice their original size within an hour followed by continued swelling to at least four times their original size over 9–12 hours. No significant difference in drug release was observed between a modified apparatus 1 with a bolus basket and apparatus 3 dissolution, ensuring formulation robustness. Based on results collected with apparatus 1 with a bolus basket and apparatus 3, the drug release mechanism from the examined formulations was determined to be mediated by erosion. Typically, GR formulations are dosed with food to replace high-intensity housekeeping waves with low-amplitude force contractions and to avoid premature gastric emptying. To simulate this, fed- and fasted-state stimulated gastric fluid (FeSSGF and FaSSGF, respectively) were used to understand the alcohol-induced, dose-dumping risk for given formulations. The formulations were found to be robust to alcohol-induced dose dumping. The performance of GR formulations was evaluated in a flexible, clinical-study design paradigm for the determination of PK and in vivo gastric retention times via scintigraphy imaging. Preliminary proof-of-concept clinical data demonstrated prolonged gastric retention of up to approximately 14–16 hours and met critical PK parameters for GR formulations as predicted by the in vitro methodology.

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DISSOLUTION OF DRUG PRODUCTS CONTAINING NANOMATERIALS

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After years of research, nanomedicines have become an emerging area in pharmaceutical drug development and have caught the attention of regulatory agencies around the globe. Recently, the Center for Drug Evaluation and Research of the US FDA published current trends in dossier submissions, pointing out that more than 50% of next-generation nanomedicines are either liposomal or nanocrystalline drug products with a considerable number of generics being under development.

Unfortunately, conventional in vitro drug release methods do not allow a meaningful assessment of the in vitro performance. Common techniques such as filtration, ultrafiltration, or centrifugation often involve high shear forces that can trigger drug release or, when reducing the mechanical stress put on the formulation, result in a less efficient separation. During the workshop on the new advancements in in vitro performance testing, some alternatives were discussed in more detail including the application of ultrafiltration to determine aqueous solubility and release of nanaocrystals. Under the conditions applied, high concentrations of stabilizers in the formulation may lead to an overestimation of the free fraction due to the formation of micelles or polymer complexes. This is also reflected by the capability of these assays to predict the in vivo situation which strongly

varies between different drug molecules. Another approach reported in literature, the quantification of release kinetics by asymmetric field-flow fractionation (AF4), uses a liquid carrier to separate the particle fraction from complex matrices. After sample collection, the colloids are exposed to this liquid carrier for the time of the separation. This reduces the resolution of the method (on the time axis) to the total elution time. For a release study carried out with liposomal carriers, an elution time of 60 minutes was reported. Also, due to the technical requirements of the method, the liquid carrier is limited to aqueous buffer media, which may significantly differ from the physiological environment.

Today, dialysis methods are the gold standard for testing the drug release from liposomal drug products. The sensitivity of dialysis methods widely depends on the rate of the membrane transport between the donor and the acceptor compartment. The surface area, the concentration gradient, as well as the thickness of the diffusion layer all have an impact on the rate constant of this transport. The limitations of dialysis as an analytical method mandate the quantification of this membrane transport, for example, by dialyzing a solution of the drug. A normalization of the experiment using this reference experiment enables the comparison of release profiles measured with different membrane pore sizes. In addition, it can be used to compare different batches of dialysis membranes before use in QC applications.

In literature, the dialysis-bag method has been applied either in combination with USP apparatus 2 or 4 USP. The formation of a diffusion layer inside the dialysis tubing often results in a prolonged release profile which is driven by the membrane transport. Also, the exact location of the dialysis beg inside the vessel can have a strong impact on the reproducibility of the measurement.

Recently, the flow-through cell was successfully used to discriminate batch-to-batch variations of amphotericin B-loaded liposomes using a dialysis process in combination with aqueous buffer media. Unfortunately, the technical setup of apparatus 4 also leads to a precipitation of proteins which does not allow the testing in presence of a protein background. Serum proteins are often involved in the release process of injectable nanocarrier formulations and potentially lead to an improved simulation of the physiological environment.

The dispersion releaser (DR) is an adapter which can be used in combination with USP dissolution Apparatus 1 and 2. The dissolution vessel represents the acceptor compartment, and the donor chamber is formed by a cage in the center of the vessel. Membrane permeation is actively supported by a paddle stirrer in the donor chamber. With this setup, the rate of membrane transport leads to a much higher sensitivity of the measurement. Recent studies include a direct comparison with conventional dialysis carried out either by using the paddle apparatus or the flow-through cell. The dialysis process was considerably faster when using the DR technology. Also, the system has been used to correlate the in vitro dissolution rate to the human PK profile of injectable nanocrystals and liposomes. In both studies, the release was investigated in presence of serum proteins.

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A NOVEL APPROACH TO DEVELOP A PERFORMANCE TEST FOR SUPPOSITORIES

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Suppositories are unique, semisolid dosage forms. Very few monograph performance tests are official for suppositories. Researchers have developed several different types of tests suitable for suppositories:

- Placement of suppository in a flask or beaker containing appropriate receiving medium
- Using USP apparatus 1 or 2 with appropriate modifications as necessary
- Utilizing membrane-controlled diffusion where the suppository is placed in a sample chamber separated by a membrane from a reservoir
- Membrane controlled diffusion where a suppository is placed in dialysis tubing, which is then immersed in a receiving medium
- Use of USP apparatus 4

The process of suppository dissolution involves the following steps:

1. Softening, followed by melting, of the suppository at the body temperature.

2. Melted suppository forms a variable mass unless controlled by a defined shape apparatus.
3. Release of the drug occurs from the melted mass into body fluids or receiving medium.
4. Transfer/diffusion of the drug from fluids across the membrane.

Three case studies were presented where a dissolution test was developed for each suppository dosage form. These tests were developed using (1) USP apparatus 1 with Palmieri basket, (2) USP apparatus 2 with sinkers, and (3) vertical diffusion cells.

In case of examples (1) and (2), the dissolution test was used as a tool in quality-by-design paradigm and the results were used to select appropriate clinical candidate formulation from many different prototypes. In case of example (3), vertical diffusion cells were used to compare dissolution of the drug from an existing gel formulation versus a suppository formulation.

DISSOLUTION OF STENTS – HOW TO DEAL WITH THE BLOOD VESSEL WALL?

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In vivo drug release profiles are a helpful tool for estimating the efficacy of implants. This approach is often not suitable for drug-eluting stents (DES) due to the small amounts of drug combined with the fact that drug concentration in the blood must not be expected to reflect the tissue concentration. Therefore, there is a distinct need for a predictive, in vitro dissolution test. Although DES have been extensively implanted for years, to date, no dissolution method, especially one designed for DES, has been described in the pharmacopeias.

Several test methods that have been used for controlled-release parenterals might be employed in the dissolution testing of DES such as sample and separate-methods, dialysis methods, the reciprocating cylinder (USP apparatus 7), and flow-through setups (USP apparatus 4). These test methods are only partially suitable for dissolution testing of DES as most of them are conducted in stirred media whereas DESs are implanted into blood vessels and diffusion of the drug into the tissue plays a major role in drug transport. Therefore, a special dissolution test method for DES should be developed that can take the distribution of the drug into the tissue wall, as well as the clearance from the site via the blood, into account.

An attempt towards a more biorelevant dissolution method for DES is the vessel-simulating, flow-through cell (vFTC), which consists of a slightly modified compendial flow-through cell for tablets (apparatus 4) equipped with a hydrogel, mimicking the tissue of the vessel wall. DES can be expanded prior to dissolution testing inside the lumen of the hydrogel and perfused with the release medium, for example, in a closed loop system. Different gelling agents (calcium alginate, agarose, polyacrylamide, and polyvinyl alcohol) for the hydrogel have been investigated. A 2% (w/w) agarose hydrogel especially was found to be very stable and easy to handle. As the hydrogels are very hydrophilic, which does not resemble the composition of the blood vessel wall, hydrophobized hydrogels were developed by adding amphiphilic or hydrophobic compounds such as lecithin, LiChroprep RP-18 or Lipofundin, resulting in a higher amount of sirolimus distributed into the hydrogel during dissolution testing in the vFTC.

Comparison of the dissolution behavior of model-drug-coated and commercially available stents between incubation setups with different release media volumes and dissolution vessel geometries in the reciprocating cylinder, a compendial flow-through cell, and the vFTC indicated a slower release of drug in the flow-through cell, particularly in the vFTC. Thus, it might be assumed that release from stent coatings is strongly influenced by the applied embedding and flow conditions and a specialized dissolution test method might be helpful for the estimation of the in vivo drug release.

Apart from the distribution of the drug into a second compartment, some other aspects have to be considered when developing a dissolution test setup for DES. Firstly, the final product should ideally be tested since surrogates such as films may provide misleading results. Secondly, the stents should be expanded, since cracking of the coating and subsequent drug loss might occur. Additionally, materials for the setup must be chosen carefully, as it is known that drugs such as sirolimus and paclitaxel tend to adsorb to the surfaces of commonly used materials such as polyvinyl chloride (PVC), silicone, or polypropylene. Another aspect that must be considered is the composition of the release medium. Frequently, PBS or normal saline is used. The usually applied drugs exhibit a low solubility in aqueous media and the use of small media volumes might lead to violation of sink conditions. In order to overcome this problem and to prevent adsorption to the surfaces of the materials, surfactants are often added to the release medium, e.g., sodium dodecyl sulfate (SDS)

or polysorbate 20 (Tween), which can also accelerate the drug dissolution rate.

Instability is a major issue for these drugs as well. For example, instability of sirolimus in buffered media is reported, but this is probably mainly due to the use of buffered media at pH 7.4. Lower pH values might lead to a prolonged stability of sirolimus, although they are probably not biorelevant. Since stability in the commonly used PBS (pH 7.4) is poor as well, stabilized normal saline (0.9%) with a surfactant (0.05% polyoxyethylene [23] lauryl ether) and butylated hydroxytoluene (BHT) (0.0003%) as an antioxidant can be recommended as release medium.

IN VITRO PERFORMANCE TESTING FOR TRANSDERMAL, TOPICAL, AND INTRA-VAGINAL DOSAGE FORMS FROM A BIOPHARMACEUTICS REVIEW PERSPECTIVE

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Dermal drug products (DDPs) encompass both dermal (local) and transdermal (systemic) products. Intra-vaginal rings (IVRs) are drug-releasing devices, generally fabricated from thermoplastic polymers or silicone elastomers, used to administer pharmaceutical drugs to the human vagina for periods typically ranging from 3 weeks to 12 months.

Tests for DDPs and IVRs are divided into two categories: those that assess general product quality attributes, and those that assess product performance, e.g., in vitro release of the drug substance from the drug product. Taken together, quality and performance tests are intended to ensure the identity, strength, quality, purity, comparability, and performance of dermal drug products.

USP general chapter <724> describes IVRT apparatuses for TDS, whereas <1724> and scale-up and post-approval change semisolids (SUPAC-SS) describes the apparatuses to use for IVRT for semisolids. There is no universally accepted method for testing in vitro drug release for IVRs. It depends on the ring types. Literature reported different types of incubators commonly used for IVRT of IVRs. Only one non-compendial shaking incubator method (for the estradiol-releasing ring, Estring) is described in the US FDA's dissolution methods database.

In general, an IVRT should be simple, reliable, reproducible, discriminating, and robust while releasing as much drug as possible and being responsive to physicochemical changes in drug products. IVRT serves as a valuable tool for the demonstration of comparative in vitro drug release rates between the test and reference products, i.e., "product sameness" during product development, scale-up, and post-approval changes; IVRT alone is not generally a surrogate test for in vivo bioavailability or BE. The new drug application (NDA) or abbreviated NDA (ANDA) submissions should include a method development and validation report with complete information and data supporting the proposed drug-release method and acceptance criteria.

Release-method development should start early in the investigational new drug (IND) phase for all submissions to propose release and stability specification in the NDA submission. Beside its role as a tool for product development and QC, IVRT method and acceptance criteria can help to bring a discontinued product back to the market in absence of a current reference product.

IVRT acceptance criteria should be clinically relevant based on a safe space built on demonstration of BE between upper and lower proposed bounds. Safe space may be built based on PBBM or IVIVC. IVRT specifications should be set based on the characteristics of batches tested in pivotal Phase 3 clinical trials. The specification time points should cover the early (burst effect), middle, and late stages of the release. The acceptance criteria range for each specific timepoint should be based on the mean percentage of drug released ± 10% using the drug release data generated at these times. A wider acceptance criteria range for the drug release test may be acceptable if they are supported by an approved IVIVC model. For TDS, an IVPT may be useful comparing the cutaneous PKs of a drug from the test and reference products using excised human skin with a competent skin barrier mounted on a qualified diffusion cell system.

IN VITRO PERFORMANCE TESTING OF TOPICAL AND TRANSDERMAL DRUG PRODUCTS: A PRODUCT QUALITY PERSPECTIVE

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Clinical, end-point evaluations of ocular, nasal, and dermal formulations are challenging due to the complexity of the formulations and the special interactions between the drugs and these tissues. In vitro methodologies

are recommended by US FDA as alternatives to in vivo studies to evaluate BE between complex generic drugs and reference listed drugs (RLDs). Recommendations in the product specific guidance for industry were based on years of research findings in method developments and data analysis. Examples of in vitro methodologies applied in studying the performance of topical and TDS are discussed below.

Rheological properties of semisolid, acyclovir topical products were measured using a stress-controlled hybrid rheometer (Discover HR-3, TA Instruments, New Castle, DE, USA). In reference to Zovirax topical cream (RLD), formulations prepared with controlled process variables did not show significant differences in yield stress and viscosity. Formulations with the same physicochemical properties were found to perform equivalently with the RLD, which was confirmed by the amount of acyclovir retained in basal skin layers observed from an IVPT. On the contrary, concentrations of acyclovir and mineral oil in petrolatum-based ophthalmic ointments were inversely related to the storage modulus of the ointments but were proportional to the in vitro release of acyclovir.

Using the fiberoptic probes integrated with USP dissolution apparatus 2, in vitro drug release from an array of ophthalmic ointments of acyclovir was investigated. It was found that the drug release rate has a linear relationship with the logarithmic scale of time instead of the square root of time as used in the Higuchi model. Therefore, a transient boundary model for drug release from petrolatum-based ointments was proposed and verified using µDISS Profiler equipped with fiberoptic ultraviolet probes (Pion, Inc., Billerica, MA, USA).

Using Franz diffusion cells with spherical joints (PermeGear, Inc., Hellertown, PA, USA), in vitro, transcorneal permeation of acyclovir from the above-mentioned ophthalmic ointments was studied. It was found that drug permeation through a rabbit cornea depends significantly on drug concentrations in the ointments. A linear relationship between the cumulative drug permeation and the square root of time was found (following the Higuchi model).

Automated diffusion cells (Teledyne Hanson Research, Chatsworth, CA, USA) were employed to obtain in vitro data for the development of transdermal IVIVC (in vitro-in vivo correlation). Level-A correlation was constructed using the data of cumulative IVPT and in vivo absorption of estradiol following the application of matrix-type TDS (patch). A strong correlation was obtained, and the model was validated. In vivo plasma concentration of estradiol was successfully predicted using in vitro data obtained at low, medium, and high strengths.

Using chambers (Physiologic Instruments, Inc., San Diego, CA, USA) loaded with EpiAirway nasal tissues (MatTek Corp., Ashland, MA, USA) were applied to evaluate nasal permeability of naloxone. Composition of excipients (preservatives and stabilizers) and pH in nasal-spray formulations were found to significantly influence the apparent permeability, transepithelial electrical resistance (TEER), and stability of naloxone.

It is worth noting that in vitro testing methods and guidance are constantly evolving with the advancements of modern technology. For example, advances in spectral imaging has provided a powerful tool for in vitro physicochemical characterization for TDS. These tools are particularly powerful for the analysis of systems that are heterogeneous in nature (i.e., crystals or different phases and excipients in a topical cream) or that can become heterogeneous after manufacturing (i.e., crystallization in TDS).

Raman mapping and multivariate image analysis was used for robust crystal identification in both commercially available fentanyl TDS with off-label modifications and in-house manufactured testosterone TDS where the effect of drug crystallization on the in vitro performance and stability was studied. By taking a spectral matching approach such as hit quality index to the modeled components extracted from the multivariant analysis, rapid identification of the crystals and other observed components could be performed.

These techniques were also applied to determine emulsion types in topical sunscreen formulations as well as to reveal micro-structures that were unable to be optically resolved. Further physicochemical analysis was performed by applying cryogenic scanning electron microscopy to the topical formulations to determine globule size distributions for correlation with drug release and skin permeating efficiency.

DISSOLUTION METHODS FOR ORALLY INHALED DRUG PRODUCTS

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Interest in developing methods to evaluate the dissolution behavior of OIDPs has developed, in contrast to oral dosage forms, only recently. One of the reasons for this delay might have been that it was recognized rather late

that dissolution step is relevant for the performance of an OIDP, e.g., by modulating the degree of pulmonary targeting.

Current methodologies employ generally a two-step process, which includes the collection of a relevant, inhalable fraction during the sample preparation, followed by the actual dissolution test. For sample preparation, drug delivered from dry powder or metered dose inhaler devices are fractionated by using, as an example, anatomical mouth-throat replicas, that allow only the inhalable fraction of the delivered dose to pass through, while larger particles will deposit in the mouth/throat replica. The respirable fraction will consequently be collected on filter paper and further evaluated within the dissolution set-up. Alternative methods have used material deposited on defined stages of Andersson or next generation cascade impactors. The Transwell system has been proposed as a means to allow dissolution under volume-limited conditions as observed in the lung. Due to the additional diffusion step and the limited volume, and contrary to the standard USP system, Transwell systems often show a dose dependency of the dissolution profile, as dissolution often occurs under non-sink conditions. Mean dissolution times observed with such systems for a range of corticosteroids have been shown to correlate well with pulmonary mean-absorption times. Application of the developed dissolution methods showed reproducibility and sensitivity in demonstrating the effects of formulation factors on dissolution of OIDPs.

DISSOLUTION OF A FINE PARTICLE FRACTION FROM A TRUNCATED ANDERSEN CASCADE IMPACTOR WITH AN ENHANCER CELL

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Dissolution testing is a key analytical test for detecting physical changes to an API with the potential impact on its biopharmaceutical properties. Thus, dissolubility determination will help in drug product development when optimizing the drug formulation and subsequently validating biopharmaceutical performance in vitro.

To date, there is no established protocol available for dissolution testing of inhaled drugs. Dissolution testing for inhaled actives is usually a two-step process: dose collection followed by the actual dissolution testing. Dose collection poses the greatest challenge for researchers as current size classifiers such as the next generation impactor (NGI) and Andersen cascade impactor (ACI) are only capable of separating the inhaled particles into various size fractions collected on separate stages. As these micronized, inhaled actives are usually very cohesive, they are difficult to collect and transfer for dissolution testing. Detachable inserts have been used together with the NGI to allow for easy removal of drug collected on a collection stage. The use of inserts only allowed dissolution testing of particles trapped on a single stage at any one time.

Multiple dissolution methods have been investigated for inhaled solids, ranging from the USP apparatus 1 and 2, to flow-through cell and Transwell systems. The lungs have very limited alveolar fluid and, therefore, a dissolution system with a small media volume would be more congruous.

A novel method of dose collection was introduced by collecting the full fine particle fraction (FPF) on a single stage and using a truncated ACI system. The truncated ACI consisted of three stages, allowing for the collection of the full FPF on a single stage comprised of a polytetrafluoroethylene (PTFE) funnel and a small collection plate. Through the optimization carried out, it was determined that an airflow rate of 60 L/min, pressure drop of 4.0 kPa, and the intermediate stage was best for the truncated ACI. The combination of the particles collected on the PTFE funnel and small collection plate made up the full FPF, which was found to be comparable to the FPF collected using the full ACI. Dose recovery was more convenient when collecting from the PTFE funnel and small collection plate. The dose collected was held in place using an enhancer cell and placed in a 200-mL, round-bottom vessel containing 50 mL of simulated lung fluid.

Although more work is required to further optimize the design of the truncated ACI system to accommodate different air flow rates and inhaler design, the study attempted to collect the full FPF for the purpose of dissolution testing. Utilization of the full FPF for dissolution testing will eventually be a necessity as it represents the pharmacologically active dose received by the patient.

ASSESSING DISSOLUTION OF ORAL TABLETS USING AN ARTIFICIAL STOMACH DUODENUM APPARATUS

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The development of a quality pharmaceutical tablet product of BCS class II drugs requires the understanding

of dissolution in human GI tract (GIT). Because of the challenges of high variability among test subjects and high cost, the use of clinical studies is not suitable for guiding tablet formulation development and optimization. An artificial stomach and duodenum (ASD) apparatus is capable of mimicking GIT physiology relevant to drug dissolution, such as pH change, liquid and particle transport, secretion of gastric and intestinal fluids. By accounting for possible complex phenomena induced by those changes, an ASD can be an extremely useful tool to guide formulation development and optimization of tablets with robust in vivo dissolution and bioavailability.

The potential benefits of the ASD apparatus in understanding dissolution behaviors of drugs are illustrated with an acid drug (indomethacin, pKa = 4.5) and a basic drug (Erlotinib, pKa = 5.42). Indomethacin does not dissolve to a significant extent in the stomach chamber over the typical pH range of 1.2–3 due to its low solubility in acidic, aqueous media. The dissolution behavior in duodenum chamber is sensitive to the pH of the gastric fluid. A more acidic gastric fluid (pH = 1.2) leads to a significantly lower dissolution rate in duodenum than a less acidic gastric fluid (pH = 2). This is attributed to the more significant pH depression in the duodenum by the pH 1.2 medium transferred from the stomach. Although Erlotinib fully dissolves in stomach (pH = 1.2), the AUC of the dissolution curve in the duodenum is sensitive to gastric secretion rate because a faster gastric secretion leads to a lower pH in the duodenum and less precipitation.

The normal pH variations in both stomach and duodenum can lead to profound variations in dissolution behaviors of both acid and base drugs due to precipitation of drug in the dynamic pH environment of human GIT. Such phenomena are difficult to capture using the single chamber USP dissolution apparatus. Oral formulations of either acid or base drugs should be development with this pH sensitivity in mind in order to attain robust biopharmaceutical performance. Compared to in vivo studies and other more sophisticated in vitro dissolution apparatus, ASD is an economical and efficient tool for routine laboratory applications to assist with this effort.

UPDATES ON IVIVC

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USP general chapter <1088> was first published in 1987. Its second version became official in 2013. The third version is under revision and is published in the *USP Pharmacopeial Forum* (www.usppf.com) for comment. This chapter describes the in vitro characterization of drug substances and drug products as well as their in vivo evaluation. The link of the in vitro to the in vivo performance of drugs is the IVIVC. It may be defined as a predictive, mathematical model describing the relationship between an in vitro property of a dosage form (usually the rate or extent of drug dissolution or release) and a relevant, in vivo response (e.g., plasma drug concentration profile).

The USP Dosage Forms Expert Committee has taken the responsibility to revise the chapter and has majorly dedicated the scientific work to a subcommittee parallel to the revisions of the related general chapters <1090> "Assessment of Drug Product Performance – Bioavailability, Bioequivalence, and Dissolution" and <1092> "Dissolution Procedure: Development and Validation."

The new proposal sharpens the focus on those characterization activities that are directed toward the goal of achieving an in vitro in vivo correlation (IVIVC). The chapter emphasizes evaluations of oral products. In addition to the glossary provided by the revised chapter, some other terms require definition.

In Vitro Characterization: Dissolution Testing

The experiments are undertaken as a topographical characterization of the drug product under various experimental conditions prevailing in the human GI tract. These include the pH-value, osmotic pressure, agitation, and surfactants. From the experimental design, constant conditions along one dissolution run are preferred to variations along the time course of the experiment. In order to ascertain the transfer to QC testing procedures, pharmacopeial apparatus are preferred. No preference is given to closed (e.g., USP paddle apparatus) versus open systems (e.g., USP flow-through cell apparatus). A sufficient number of data points must be collected for a statistically meaningful sample size (i.e., $n = 12$). Variations of one drug product must be examined with different compositions and/or after changes of critical material attributes and critical process parameters.

In Vivo Evaluation

The in vivo evaluation of dosage forms requires a PK profiling of the drug substance first. The classical disposition parameters are of primary interest. These include oral bioavailability, volume of distribution, and elimination kinetics. Active metabolites and enantiomers must be known as well as manipulations of the drug substance undertaken, e.g., following the

prodrug concept. Phenomena such as enterohepatic cycles, absorption windows along the gut, or instability of the active moiety in the GI environment are of great importance besides the existence of linear PKs. The effect of age, gender, and race may be considered as well as chronopharmacokinetic effects; these should be known before the product's bioavailability is investigated. The drug product of interest may be an immediate release dosage form or a modified release dosage form, where the release is either delayed, extended, or both. The correlate to the in vitro dissolution and drug release kinetics are deconvoluted from the plasma-level versus time profiles. Usually, they are not directly accessible in the case of oral dosage forms. The design of the in vivo investigation is mostly identical to a classical bioavailability or BE study and may be integrated into such a study. The investigation of an oral solution is considered to provide the refence of choice, if feasible. Otherwise, a well-characterized, immediate-release dosage form may serve as reference. The blood-sampling grid must be adapted to the need of describing best the invasion phase as well as the individual elimination kinetics for the subjects involved in the study. They are preferred to literature values.

IVIVC

The IVIVC is a predictive mathematical model. In an ideal case, the function is linear. The IVIVC is used to predict differences in bioavailabilities of dosage forms using their dissolution data. An in vivo-in vitro relationship (IVIVR) simply states that an in vitro change will result in an in vivo change, but the amount of change is not mathematically predictable.

Depending on the amount of data used to compute the IVIVC, there is a rank order established. Level A correlation uses entire in vitro and in vivo profiles. The in vivo release kinetics are either computed by linear systems analysis as algebraical or numerical deconvolution or are based on PK models such as Wagner, Nelson or Loo, or Riegelman. The level B correlation is based on the statistical moment theory. Although kinetic data are reduced to one or a few parameters its advantage is, that it may be used in cases of non-linear PKs. Level C correlation is considered to provide the lowest level of information using the classical disposition parameters such as AUC or C_{max} to correlate with the amount dissolved in vitro at a certain time. To establish an IVIVC requires the proof of its predictability. Establishing a correlation does not necessarily require a biorelevant dissolution method. In the case of immediate release dosage forms, being categorized as BCS class II, and under certain circumstances also class IV, biorelevant media may be advantageous. In the case of extended release dosage forms, which, in an ideal case, release the drug independently from their physicochemical surrounding, biorelevant media may not be needed.

The revised general chapter <1088> is of great use in the development of oral dosage forms. Its glossary provides clarification with the so-called buzz words in biopharmacy.

QUESTIONS AND ANSWERS

The answers have been provided by the corresponding speakers and other speakers may not agree with them.

- **"First-Principles Approaches and Surrogate Testing for Predicting In Vitro Dissolution"** by *Andre Hermans, Merck & Co., USA*
- **"Three-Dimensional (3D) Printing for Fast Prototyping of Pharmaceutical Dissolution Testing Equipment for Nonstandard Applications"** by *Przemyslaw Dorozynksi, Instytut Farmaceutyczny, Poland*
- **"A Systematic Approach to Develop Predictive Dissolution Models"** by *Fernando J. Muzzio, Rutgers University, USA*
- **"Dissolution Modeling for Real-Time Release Testing (RTRT)"** by *Hanlin Li, Vertex Pharmaceuticals, USA*
- **"Development of Real-Time Release Test of Tablet Dissolution"** by *Sarah Nielsen, Janssen Pharmaceuticals, Puerto Rico*

Q The dissolution modeling for RTRT seems to be applied only to tablets and may be also to modified-release tablets. How does it apply to other dosage forms?

A There is no reason why one could not apply the same modeling to any other dosage forms. Probably other factors or attributes may need to be considered, but the same concepts are involved regardless of the type of dosage form. It is probably limited by the continuous manufacturing equipment as currently most of the platforms are designed only for solid oral dosage form that are the most popular.

Q It was said that it is possible to make a more discriminating test for smaller particle sizes. When developing a test for RTRT, how can it be demonstrated

that the most appropriate in vitro test is being used?

A The foundation of RTRT testing comes from the reference model. It is addressed first using the traditional dissolution test and it needs to have the appropriate discriminative power.

Q **For companies that are just thinking about RTRT, what skill sets are needed?**

A The special skills needed include PAT, spectroscopy, and chemometrics. Also, subject-matter experts can help in the development of the model. The PK and pharmacodynamic (PK/PD) modeling is handled by people with a background in pharmaceutics.

Q **What is the sampling strategy in continuous manufacturing? How many segments are sampled and what criteria are applied?**

A Each hopper is sampled, and the mean standard deviation has to be less than what is called by the model.

Q **What factorial design is used?**

A Most of the time, the fractional factorial is used.

- **"Biorelevant In Vitro Gastrointestinal Model (tTIM-1) for Food-Effect Prediction"** by *Shirlynn Chen, Boehringer Ingelheim Pharm Inc., USA*
- **"The Biorelevant Gastrointestinal Transfer (BioGIT) System for Assessing the Impact of Dose and Formulation on Early Exposure After Oral Administration"** by *Christos Reppas, National and Kapodistrian University of Athens, Greece*
- **"Dissolution for Products Applied to the Oral Cavity"** by *Sandra Klein, University of Greifswald, Germany*
- **"Utilizing Conventional Dissolution Technique to Predictive and Guide Successful Development of Gastroretentive Drug Delivery Systems"** by *Sanjaykumar Patel, Merck & Co., USA*

Q **Why does the gastric retention formulation have large variability in retention time?**

A The initial variability is due to lag time. The variability seen on day 1 should go away the next day because of the concentration of the drug in the body.

Q **Some orally applied dosage forms are designed for quick absorption, like products to treat nausea. How do you have a more physically relevant dissolution test?**

A Sampling is very important for orally applied dosage forms that dissolve rapidly. If a reliable number of samples cannot be taken in a short period of time, like a minute, dissolution testing is not an appropriate test. The same applies to oral films.

Q **Is there a proper animal model for gastroretentive dosage forms? How are you sure that your formulation is retained?**

A There are no animal models that can mimic the human body for this purpose, so understanding the formulation properties is more important. The gastric retention was confirmed with the gamma scintigraphy imaging. The swelling of the dosage form is critical, this is how we know it is retained.

Q **Have you compared the results obtained with the tiny TNO gastrointestinal model (tTIM-1) in a transfer model from gastric medium to intestinal medium?**

A Yes, we have done the comparison for the fasted conditions. The problem with the transfer model is the absence of enzyme and there are no dynamics. We did not obtain a good correlation with the in vivo model. We tried to use a conventional two-step model using medium containing bile, but there is the limitation of not being able to generate sink condition. The transit and hydrodynamics cannot be duplicated. It was even worse with the food effect. We obtained better results with fasted conditions.

Q **Which simulated saliva was used with the dosage forms applied to the oral cavity?**

A The medium composition was not very important in our lozenges' experiments. However, the media selection strongly relates to the type of formulation and you need to know your formulation very well to decide for an appropriate medium. In other experiments of the same type, you may need to add enzymes. For lozenges, a very simple medium can be used. My advice is to first perform a simple dissolution test using "basic" conditions (i.e., a simple aqueous buffer with a certain pH). Then, stepwise, add additional ingredients that might be relevant and

check whether they have an impact on dissolution/drug release or not. If yes, the medium will need to closely resemble saliva composition and properties, if not, you may even use water for the dissolution experiment.

- **"Dissolution of Drug Products Containing Nanomaterials"** by *Matthias Wacker, National University of Singapore, Singapore*
- **"A Novel Approach to Develop a Performance Test for Suppositories"** by *Kailas Thakker, Tergus Pharma, LLC, USA*
- **"Dissolution of Stents – How to Deal with the Blood Vessel Wall?"** by *Katharina Pruessmann and Anne Seidlitz, University of Greifswald, Germany*
- **"In Vitro Performance Testing for Transdermal, Topical, and Intravaginal Dosage Forms from Biopharmaceutics Review Perspective"** by T*apash K. Ghosh, Division of Biopharmaceutics, Office of New Drug Products/ Office of Pharmaceutical Quality (OPQ), Center for Drug Evaluation and Research, US FDA, USA*
- **"In Vitro Performance Testing of Topical and Transdermal Drug Products: A Product Quality Perspective"** by *Yang Yang, Division of Product Quality Research, and Daniel Willett, Division of Pharmaceutical Analysis, Office of Testing and Research, OPQ, CDER, US FDA*

Q The incubating shaker methods are very commonly used in companies that develop vaginal dosage forms. Years ago, some people from US FDA said they don't want to see these methods anymore in product applications. We do a lot of work on vaginal dosage forms and we are working on more biorelevant methods, particularly to try to explain birth defects. We are approaching more predictive tests methods and wondering if this is what the FDA would like to see.

A At the FDA, we do not generate data most of the time, you are our ears and eyes, so we will look at whatever you produce. That's how we gain knowledge and understanding. If you develop the method properly, generate enough data, and have proper validation, we do not say that the method is not right unless there is an obvious reason to say that.

Q The transdermal dosage forms could be amenable to a study that would elucidate the properties that actually control drug release, enabling us to move away from release method towards the predictive model for release. For vaginal ring systems, the 21-day release test may not be a very commercially friendly approach. Is there any encouragement at the FDA to the use of more predictive models?

A In vitro release testing and predictive models are the wave of the future; we are encouraging these models a lot. So far nothing has been proposed, but we are learning and hiring more people with relevant training.

Q What is the particle size cutoff for the filtration of products containing nanomaterials? How to better separate the particles from the free drug?

A It depends on the formulation. With nanocrystals, we did not obtain good results with 0.1-µm filters. We tried to confirm the results with nanoparticle tracking analysis (NTA), one of the particle size measurement tools. We tried to confirm that no particles permeated the filter membrane, but when so much pressure is applied to the filter (as with a syringe filter), a few particles typically go through the filter. Keep in mind that particle size is also decreasing during the dissolution test, which is the whole idea of the test.

Q When we develop a dissolution method, we want it to be discriminating, but some of the properties are very dependent on pH and temperature. If we change the temperature by 1 or 2 degrees, we lose our discriminating power. How do you balance the trade-off?

A For transdermal or intravaginal forms, "dissolution" is a misnomer: it is preferable to use release test. Developing a discriminatory method can fill a lot of gaps. Otherwise, every time a change is made to a formulation or manufacturing process, you have to go back and start all over again. If a company invests time and money at the development stage, this will prevent a lot of problems in the future. You know your product better than anybody else, and you have to handle the trade-off. When you intentionally alter some of the parameters, you can see how this affects the release methods.

Q Any comments on the separation of nanoparticles for solubility studies?

A The literature shows that, frequently, the separation procedure will depend on the formulation. For example, a nanocrystal is often very stable, and you may be able to achieve a very good separation, but if it is a liposome, the outcome is unpredictable. When you achieve the appropriate particle collection, you should confirm the results with a second method or try to do a size measurement, which is difficult. NTA can be used. Another option is to quantify the drug after the initial filter separation step to make sure there are no remaining particles.

Q The use of light scattering was mentioned for particle size determination. What about looking at light scattering as a surrogate for measuring drug concentration? You could follow loss of turbidity as a surrogate for dissolution, for particles disappearing.

A There are two problems with light scattering: it is not a robust system because you will always see interference from the medium. The second problem is that light scattering is an optical measurement that mostly depends on how your particle surface behaves, it is far from being as sensitive as other quantification methods. As a second measurement it might be good, but it should not be considered as the first method.

Q For nanoparticles it is understood that the critical parameter is the count concentration and not mass concentration. How to get mass concentration for the dissolution?

A Light scattering is not used for the quantification of the drug. It is used to see if the filtration is reducing the particle count. It gives us a cutoff where we do not find particles anymore, it is a qualitative measurement. It is not used for the direct quantification of the particle count that is later translated into a dissolution rate. With NTA, only certain concentration ranges can be measured, depending on the material that is being tested.

Q Have you already tried to correlate the count concentration with flow fractionation?

A So far, we have not. It is certainly something we would like to do to have a more accurate determination of the particles. I do not really consider field-flow fractionation to be a suitable measure of the kinetics, because later you cannot ever run the field-flow fractionation in the same sample. Rarely, you can run it in the same medium. This means that the duration of the separation run is also a time span when you get your formulation to release more, which means the resolution in the dissolution test is the run time of your field-flow fractionation. So, it is a separation method that does not stop the formulation from releasing and that is a problem.

Q Can you do it very fast?

A It is a problem. Let's say you can reach a run time of 3 or 4 minutes. This breaks down the resolution of the dissolution test to these 3 or 4 minutes and it also means that you would have to investigate the same medium as in your field-flow fractionation, and we know that method development for this kind of system is already very complex. Now you have to run it in a medium that is also good for dissolution. It is not the most practical choice. I still would like to run a study where we can separate the different particle fractions and be able to analyze what happens in a dissolution test that is made for measuring kinetics.

- **"Dissolution Methods for Orally Inhaled Drug Products"** by *Guenther Hochhaus, University of Florida, College of Pharmacy, USA*
- **"Dissolution of Inhalers"** by P*aul W. S. Heng, National University of Singapore, Singapore*
- **"Assessing Dissolution of Oral Tablets Using an Artificial Stomach Duodenum Apparatus"** by *Changquan Calvin Sun, Department of Pharmaceutics, University of Minnesota, USA*
- **"Updates on IVIVC"** by *Johannes Kraemer, DISSO GmbH, Homberg, Germany; USP General Chapters – Dosage Forms Expert Committee*

Q IVIVC is a very systematic approach, which is good. When you want to compare something to an oral solution, you need an oral solution. Very often, you have to do lots of formulation work to achieve this solution, so the question is, is that comparable? fast?

A You picked up on a limitation. Sometimes you have no oral solution. Then, you can use an immediate-release dosage form. You take into account that it is not exactly a solution, but it is as close as we can get to a solution.

Comment from one of the attendees: I have a comment about which dissolution method – the simpler one or the more complex one – should be used for QC release.

You also want to have some kind of clinically relevant, or biorelevant, feature. The important thing is first to identify the rate-limiting steps that are really controlling or contributing to absorption. It could be limited by simple particle-size dissolution, and, in that case, your simple dissolution could easily correlate with in vivo because close to 100% of absorption is how fast the drug releases and how much. However, some BCS class I and III drugs, especially the high-dose ones, become solubility-limited. In those cases, it may be difficult for the simple dissolution procedure to capture it. Maybe a transfer model can capture that, if the supersaturation step is really controlling how much of the drug is available for absorption. Focus on that step to have some in vivo predictability. You do not have to use tTIM-1, but it can help you to understand those critical steps.

Q In the USP general chapter <1088> "In Vitro and In Vivo Evaluation of Dosage Forms," do you intend to characterize different scenarios? For the immediate-release, high-dose formulation with a BCS class II drug, instead of putting in the effort to develop an IVIVC, I think it will be better to work within the safe space. This is for compounds that are not very straightforward and are more complex.

A Yes, that is exactly the intention. BCS is not mentioned in the present version of the chapter, so we brought this in because the BCS is the basis for all the related FDA guidances, such as biowaiver, SUPAC for immediate-release, and modified-release dosage forms. We have to go in this direction, to harmonize with FDA, and by the way it reflects the international state of science.