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In-Vitro Product Performance of Parenteral Drug Products: View of the USP Expert Panel

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ABSTRACT

Performance testing of parenteral products represents a broad arena of product types, test equipment, and analytical challenges. This Stimuli article is one in a series of Stimuli articles on product performance testing focused on common methodological approaches used and challenges encountered in the field of performance testing of injectable products. The article is complementary to In Vitro Release Test Methods for Parenteral Drug Preparations <1001> and takes into account the contents and acknowledges current trends in test apparatus and conditions, medium selection, and separation techniques. Limitations of current practices are presented, and recommendations highlight the need for biorelevant and predictive test environments, test standardization, and an understanding of the impact of the test conditions on the release kinetics and interpretation of test results.

INTRODUCTION

The surge in biologics approvals in the last decade has catalyzed a resurgence in the acceptability of injectable and implantable drug products especially those with infrequent administration (*1*, *2*). Research and insights into the product performance and the rational design of these drug products have also expanded. This article will serve to raise awareness of current practices and new advancements for injectables and implantable drug products. The foundation for the article is *In Vitro Release Test Methods for Parenteral Drug Preparations* <1001>, which provides selected product quality tests for common injectable and implantable drug products. This article is one in the series of *Stimuli* articles being developed by the USP Expert Panel for New Advancements in Product Performance Testing. The first Stimuli article in this series is titled *Testing the In-Vitro Product Performance of Nanomaterial-Based Drug Products: View of the USP Expert Panel* (*3*), and complements this article when injectable or implantable drug product includes nanomaterials.

While each *Stimuli* article is specifically focused on certain topics, there are some general principles related to biorelevance and clinical relevance, as well as critical parameters and system variability that should be kept in mind. When describing whether a method is intended to be bio- or clinically relevant, the following should be clarified to the appropriate level of detail: whether an aspect of the test conditions or test result (e.g., solubility or dissolution profile) is similar to that attained in vivo (clinically relevant) or whether aspect(s) of the test

conditions or test environment are similar to the in vivo environment (biorelevant). Additionally, it should be clarified which aspects of the test conditions are bio- or clinically relevant, for example, which aspects of the medium composition are biorelevant. Parameters critical to the in vitro performance/release methodology should be identified as well. For these parameters, system variability must be characterized to quantify the potential impact of variability on drug release. With respect to accelerated testing, system variability is particularly relevant to long-acting injectables and implies some divergence from biorelevance.

The current *Stimuli* article focuses on drug release test method opportunities and challenges related to: 1) apparatus, 2) accelerated testing, 3) medium selection, and 4) separation techniques. It also includes a gap analysis of methods currently in use, as described in <1001> and elsewhere in the literature for in vitro drug release for a variety of parenteral dosage forms. This gap analysis follows a systematic consideration of methodological challenges, novel methods, and recommendations for method development.

APPARATUS

There are multiple examples in the literature of both compendial and non-compendial apparatus used. Methods used can be described as sample and separate, continuous flow, or dialysis methods. An Erlenmeyer flask with orbital shaking is an example of a simple release set-up which has been used with bioresorbable polymer formulations, with regular sampling and medium replacement from the flask, to determine release kinetics of model drug compounds from the tested formulations (*4*).

Apparatus 4 (flow-through) has been used in open and closed systems, with different pump types and cell sizes allowing variations in local hydrodynamics. The cell can be varied by using beads or dialysis/implant/semi-solid adaptations (*5*, *6*). Other non-compendial continuous flow set-ups have also been used. *Apparatus 4* has the advantage of enabling a closed system which minimizes evaporation over longer testing periods with lower shear rates. However, it can present challenges with filter blockage from test samples or excipients, or precipitation of serum proteins and protein drugs, and it is important to prevent air uptake into the system. The shear forces in the capillary system as well as the use of glass beads have led to observations of visible denaturation within 24 h (*7*).

Standard configuration and reverse dialysis systems have been employed, with standard dialysis being useful to simulate conditions where the dosage form is immobilized on administration (*5*, *6*, *8*). This method also enables easier medium replacement, prevention of evaporation, and provides better sink conditions than the sample and separate methods. The sample in the dialysis bag can be agitated through rotation or a constant temperature shaker, if not via the continuous-flow method.

The stirrers of *Apparatus 1* and *Apparatus 2* have been used with the dispersion releaser, assessing release from liposomes, nanoparticles, microparticles, and nanocrystals (*3*, *7*, *9*, *10*, *12*). The set-up minimizes evaporation with an average weight loss per vessel of approximately 3.4% at 37° over 21 days. For other set-ups using *Apparatus 1* or *Apparatus 2* and without a suitable sampling port or isolation, significant evaporation can occur. Other less common or novel methods include the SCISSORS (Subcutaneous Injection Site Simulator) kit, where the test volume is injected into a cell cassette, with sampling from a receptor sink compartment (*13*).

Microbiological methods have also been employed where dissolution of a drug with antimicrobial properties can be characterized through pharmacodynamic assessment of microbial growth (*14*). A limitation of this approach includes potential negative effects on microbial growth kinetics of the medium used. An advantage is the potential to observe pharmacodynamically active drug release. Other approaches to pharmacodynamic characteristics in performance assessment have been explored, such as ex vivo models which show potential for performance testing of subcutaneous injections (*15*). A novel example of replication of physiological phenomena is the use of a chamber that physically compresses the drug product within *Apparatus 2*, as an exploratory approach to mimic the effects of muscle contractions around a granuloma that incorporated the product (*16*). The relevance of the administration setting should also be considered. Recently, an in vitro method has been presented simultaneously analyzing particle size and dissolution rate during dissolution testing, to explore the impact of syringe-induced shear on parenteral suspension dissolution. Such methods have the potential to determine the impact of the injection procedure on dissolution in the clinical setting (*17*).

With respect to apparatus selection and test set-up, it is recommended to consider whether the following aspects represent critical test parameters:

Agitation: Agitation conditions should be considered to ensure adequate mixing in low-velocity regions of the apparatus. Conversely, low flow conditions may be

intended to replicate certain physiological environments. In continuous flow systems, the assumption of sink conditions should be considered with caution when using very low flow rates.

Volume: Similarly, the volume of medium used should indicate whether sink conditions are being attained and if that is intended.

Temperature: Temperature is usually 37°, and if not, it is generally aimed at biorelevance, unless accelerated dissolution conditions are used.

ACCELERATED TESTING

During drug product development, in vivo predictive performance test methods are needed to ensure product quality (*18*). However, dosage forms with a very long period of drug release—months in the case of some injections or implants—may require trade-offs during the development of performance tests. On the one hand, one may wish to maintain a high level of biorelevance for the in vitro test to allow the detection of the broadest spectrum of performance changes. Usually, this means an in vitro test duration designed to closely match the timescale of in vivo drug release. On the other hand, long tests may not be practical for making decisions necessary for commercial product release. As a result, performance tests that can be performed quickly ("accelerated") are often developed with the knowledge that while they increase practicality, the differences in drug release time scale and test conditions may make it difficult to detect or interpret some potentially important manufacturing changes or defects that could adversely affect product pharmacokinetics or safety (*19*). While accelerated performance tests are still often developed for quality control purposes, these tests may offer less predictability of in vivo performance (as suggested in *In Vitro and in Vivo Evaluation of Oral Dosage Forms* <1088>) due to reduced confidence in the tests' biorelevance.

<1001> states that accelerated methods are necessary to assist in the evaluation of parenteral drug products. To date, there is no USP guidance regarding the appropriateness of accelerated test media, apparatus, or parameters for injectable dosage forms. <1088> provides descriptions of the relationships between in vivo pharmacokinetics and associated in vitro drug release methods, and *Dissolution* <711> gives guidance on dissolution testing for oral dosage forms; however, these chapters do not mention accelerated testing. Adjustments to performance testing conditions that could modify performance test run times may include:

- Assay temperatures greater than 37° (*20–22*)
- Higher paddle rotation rate for some types of dissolution test apparatus (*20*, *23*)
- Use of hydroalcoholic media or media containing surfactants or cyclodextrins (*22*, *23*)
- Choice of osmolality and ionic strength of the medium (*24*)
- Medium viscosity (*25*)
- pH changes (*26–29*)

Media used in accelerated test set-ups are often hydroalcoholic mixtures or buffers containing surfactants but typically do not contain physiologically relevant ingredients. However, if it is possible to simplify and accelerate a biorelevant release method, such as an accelerated method that can be used to predict real-time in vivo release, then accelerated methods could have biorelevance. Overall, accelerated test methods require careful validation regarding known effects, such as media evaporation. Furthermore, when using an accelerated method, it must be ensured that the release mechanism of the dosage form under investigation is not affected by the composition of the medium (*22*, *30*).

For some dosage forms such as microspheres (*10*, *21*, *28*, *31*), manipulation of in vitro test parameters to decrease test run times can still lead to tests with definable correlations between in vitro and in vivo performance, or at least a secondary relationship between an accelerated and a non-accelerated (and more biorelevant) test. A correlation could be established due to having prior knowledge of the mechanisms of drug release, in vitro and in vivo release phases, and other physical properties of the dosage form. Lessons learned from one type of modified-release injectable dosage form may not be applicable to others.

Some biorelevance aspects of performance tests may be compromised with accelerated methods, such as differences in mechanism of release from the dosage form depending on testing (in vivo or in vitro) (*21*), microenvironment of the site of administration/media (*10*), and release phases of the dosage form (*31*). Dosage form attributes that should be considered with respect to biorelevance during test acceleration may include:

- Matrix glass transition temperature (*20*, *21*)
- Solubility of the formulation components in the media (*21*)

- Microsphere polymer degradation rate (*31*)
- Intra-particle diffusion kinetics (*23*, *26*)
- Stability of the dosage form in the medium (*32*)

The Dissolution Procedure: Development and Validation <1092> and FDA guidance both point out the importance of an in vitro drug release test's ability to discriminate between an acceptable and an unacceptable batch (*33*). Often, formulations of dosage forms with different physicochemical properties such as particle size are chosen to demonstrate the discriminatory power of the associated performance test (*34*). The development of accelerated performance tests with acceptable discriminating ability also involves assessing the frequency of the various types of manufacturing failures and their risk to safety and effectiveness (*35*). It cannot be assumed that all significant failures can be detected by an accelerated performance test. Some of the barriers to reducing uncertainty and risk that should be overcome include:

- Lack of awareness of critical product attributes that may be altered during manufacturing, an aspect that an accelerated performance test may not detect
- Difficulty in determining the precise mechanisms of release and impact of acceleration on those mechanisms for novel dosage forms
- Difficulty determining the impact of sometimes extreme time-scale compression and scaling on the predictability of various types of in vivo in vitro correlations (IVIVC) identified in <1088>
- Incomplete or not completely understood mitigation strategies to reduce the uncertainty and risk of test acceleration
- Lack of standardization of accelerated performance tests

MEDIUM

Numerous media have been proposed for in vitro release testing of injectables and implants. Their composition is typically linked to the purpose of their use and ranges from simple buffer systems ensuring robust pH conditions and media including surfactants and/or hydroalcoholic media to increase the solubility of the drug substance to media intended to mimic the environment at the injection/implantation site.

As can be seen from the suggested media composition of current quality control methods for injectables and implants listed in the FDA Dissolution Methods Database (*36*), media composition and properties are strongly tied to the drug substance to be administered and the apparatus to be used. Media suggested include water with or without the addition of sodium lauryl sulfate (SLS) or polysorbate 20, acetate and phosphate buffers, and water and methanol mixtures. The media pH ranges from pH 3–7.4, and the media are used at temperatures between 25° and 47°. All these media were designed to develop robust and discriminatory quality control tests but not intended to be biorelevant.

Few studies have been performed assessing in vitro performance of intravenous injectables. Most studies focused on discriminating formulations in a reasonable time by varying media composition and temperature. One study developed a method to study amphotericin B release from liposomes by adding γ-cyclodextrin to a pH 7.4 HEPES buffer containing sucrose and NaN3 to prevent amphotericin B precipitation. This media in combination with an increase in media temperature raised to 55° enabled drug release within 24 h without affecting the liposome structure (*37*). Jablonka et al. suggested the use of phosphate-buffered saline supplemented with cyclodextrins and fetal bovine serum (*9, 38*). With the cyclodextrins acting as a solubilizer, simulating the distribution of the drug into deeper compartments, the impact of drug release on pharmacokinetics was successfully predicted. The amount of solubilizer was selected based on the pharmacokinetic parameters of the free drug and does not represent our traditional understanding of sink conditions.

For liposomes for the drug-protein transfer, a release of drug molecules from the liposome into the protein-bound fraction has been confirmed (*7*). It is an important release mechanism that impacts the distribution of the released drug. In combination with the appropriate equipment and instrumental settings, an IVIVC could be achieved with some of the media used in quality control for certain drugs. This should not necessarily be expected for other dosage forms of this type, even if they contain the same active ingredient. When aiming to understand and predict in vivo performance of injectables, it is important to properly address physiological parameters relevant to in vivo drug release. Therefore, it is essential to know the injection/implantation site. Upon injection, intravenously administered formulations are immediately diluted within the blood-stream; this will not be the case at other administration sites, such as subcutaneous tissue

or muscle. For this reason, besides the media composition and properties, the medium volume applied in an in vitro test is also an important fact to consider.

A medium for the assessment of intravenously administered formulations should, where possible, contain the blood plasma ingredients that are determined to be essential physicochemical properties that could affect in vivo drug performance (for example, water, plasma proteins, and electrolytes). To date, a simulated plasma fluid has not yet been described. Aiming to design biorelevant in vitro test methods for liposomal drug products and nanocrystals, recently published studies have evaluated new media compositions that take into account physiological pH as well as other factors such as physiological protein and surfactant concentrations, as well as plasma osmolality (*7*, *9*, *38*, *39*). In some cases, a level A IVIVC could be achieved using these media. Nevertheless, due to some of their components, such as cyclodextrin derivatives and artificial surfactants, having been most likely added to obtain sink conditions when using lower media volumes than the original plasma volume, these media cannot be considered biorelevant. Their biopredictive character originates from a simulation of release conditions difficult to mimic without further information on the behavior of the compound in the blood during circulation. Still, the way these media were designed represents an interesting platform for future media design for intravenous injectables. Once the mechanistic relationships contributing to the release of the drug from the formulation have been understood, clinically relevant media can be designed based on available clinical data for the drug candidate.

Many injectables, such as intramuscular and subcutaneous injections and implants are administered at sites that have a completely different environment, featuring different water content, proteins, lipids, and other components. At the injection site, the formulation comes into contact with tissue and, initially, very little liquid. However, the latter is in equilibrium with plasma and lymphatic fluid, ensuring drug transport. Such an environment is rather difficult to simulate in an in vitro test set-up. Furthermore, the composition of many physiological fluids is not well understood; this also applies to interstitial and muscular fluids, of which many individual components and physiological concentrations are not known.

In many studies, phosphate buffered saline (PBS) pH 7.4 is used in quality control of intramuscular and subcutaneous injectables (*40*). An alternative that might better simulate physiological phosphate concentrations is a modified Hank's balanced salts solution (*41*). While these media have been successfully used to discriminate among microsphere and implant formulations containing risperidone and naltrexone, respectively, they are not considered clinically relevant because they only take into account some of the electrolytes present in physiological fluids and pH.

Several simulated physiological fluids for the purpose of developing discriminative dissolution methods for injectables have been proposed and their potential application in dissolution testing has been discussed in review articles (*42*, *43*).

In a recent study, Simon et al. screened phosphate buffer pH 7.4, and the more biorelevant media Simulated Body Fluid pH 7.4, a phosphate-based buffer containing several other electrolytes, and Simulated Muscular Fluid pH 7.4, a saline imidazole buffer, without and with different amounts of SLS added, with the aim to develop and validate a discriminative dissolution test for betamethasone sodium phosphate and betamethasone dipropionate intramuscular suspension. The experiment was performed with *Apparatus 2* at 50 rpm, pH 7.4 phosphate buffer with 0.1% SLS added to provide the highest discriminatory power regarding the differences in particle size found between the tested suspensions (*44*). As discussed for intravenous injectables, a discriminating method is not necessarily clinically relevant. This case is an example of a method that is good for assessing the impact of critical product attributes of a given drug product, but where the proposed method is not necessarily predictive of in vivo performance for intramuscular injectables. Similar to the situation for intramuscular injectables, a clinically relevant fluid for in vitro testing of subcutaneous formulations currently does not exist.

A study by Gao et al. presented a novel Simulated Subcutaneous Interstitial Fluid (SSIF), which is a biorelevant medium designed to reflect major characteristics of the subcutaneous tissue (ionic composition, buffer capacity, and protein concentration) and was applied to a novel dispersion releaser set-up, which allowed discrimination between drug release of microparticles before and after storage (*10*). This medium can be considered as a first step towards a more biorelevant medium that could also be applied in quality control (*45*). The composition of this medium still represents a compromise between an exact reflection of the biological environment and biorelevance (*41*). To further explore the suitability of SSIF, more in vivo data will be required.

Since no large amount of free fluid is available after subcutaneous injection, when aiming to mimic the in vivo environment, media with higher viscosities are sometimes considered more biorelevant than simple aqueous fluids. In the course of developing a novel delivery system for the sustained release of biopharmaceuticals, an agarose-based hydrogel was obtained containing 2% (w/v) agarose and 10% glycerol in PBS pH 7.4. This media was promising for assessing protein release in a simulated interstitial environment (*46*). However, the method is unlikely to work for all types of implants since the focus was set on maintaining a physiological pH and a certain viscosity rather than simulating the essential composition of interstitial fluid.

Hydrogels have also been used in in vitro release testing for other groups of implants, namely drug-eluting stents used in vascular intervention. Drug substances released from these devices are intended for local action. Common drug-eluting stents are bare-metal stents coated with a polymer that contains the drug substance. Consequently, the drug substance can be released from the entire surface of the drug-eluting stent. After implantation into a blood vessel, the outer (abluminal) side of the stent is in direct contact with the vessel wall, whereas the inner (luminal) side is perfused by blood. Consequently, drug release can either occur into the designated site of action which is the tissue of the vessel wall or into the blood circulation. Determining drug release of drug eluting stents in a simple set-up, where the stent is immersed in a compendial medium will hardly be predictive of in vivo performance. Nevertheless, most of the methods reported to date use simple non-standardized incubation set-ups, *Apparatus 4* or *7*, and simple aqueous media such as saline solution, acetate buffer, or PBS pH 7.4 mixed with acetonitrile, methanol, and/or surfactants such as SLS, Tween 20, or Triton X-100 to ensure sink conditions, or bovine serum albumin to better simulate the composition of blood plasma. To simulate some of the in vivo parameters that can impact the release behavior of drug eluting stents, more biorelevant in vitro methods have been developed (*47*). The vessel-simulating flow-through cell is based on the compendial flow-through cell containing an additional compartment simulating the vessel wall, which allows for the examination of drug release and distribution (*48*). In this set-up, the vessel wall is simulated by an alginate hydrogel, whereas the flowing blood is simulated by PBS pH 7.4. The use of hydrogel was a first step towards increased biorelevance. Nevertheless, this experimental set-up does not necessarily provide clinically relevant results but may require further modifications, especially with regard to the composition of the gel compartment which could be further modified to better represent the specific components that might influence drug release and distribution into the vascular tissue. There are many options of how to further improve in vivo relevance of such method. It has been questioned if such complex test methods would be the methods of choice for quality control when more simplified methods would present sufficient discriminatory power.

A Simulated Synovial Fluid obtained by dissolving 3% (w/w) hyaluronic acid, i.e., one of the constituents of synovial fluid, in PBS pH 7.4 was developed for testing cation dissolution from glass microspheres intended for direct placement into a joint (*49*) of a rheumatoid arthritis patient. More recently, Biorelevant Synovial Fluids (BSF), containing physiologically relevant amounts of hyaluronic acid, phospholipids and proteins, meant to simulate healthy and osteoarthritic conditions in a joint, were applied in a study evaluating the performance of different controlled release formulations of methylprednisolone for intraarticular administration (*50*). Available data indicate that the composition of the BSFs, particularly the protein content, had a significant impact on drug release of the tested microsphere formulation. As for other injectables, these might be ingredients to consider in future media development when aiming to design discriminating and predictive test methods.

SEPARATION TECHNIQUES

While the vast majority of injectables are aqueous solutions, a growing number of particle dispersions, semisolids, micelles, and emulsions are administered parenterally (*51–55*). For these advanced delivery systems, the technology employed in the separation of the monomolecular drug from the excipients and medium components plays an important role (*56–59*). Depending on the exact size of the particles and the medium composition, the purification may become more challenging (*56*). Although *Injections and Implanted Drug Products* (*Parenterals*) <1> does not recommend a particle size range for dispersions, the average diameter of particles often falls into the micrometer or nanometer scale to ensure compatibility with the administration site (*51*, *53*, *55*, *63*). With decreasing particle size, the separation becomes more challenging with regards to the selectivity for a specific particle population and the sensitivity of the assay to the drug being released from the carrier. A detailed explanation of the most common separation methods is provided by the *Stimuli* article *Testing the in-vitro product performance of nanomaterial-related drug products: View of the USP Expert Panel* (*3*). Although the article emphasizes methodologies

for the testing of nanomaterial-based drug products, there are huge overlaps with the separation methods applied to microparticles and macroparticles (*10, 56, 59*). One important difference lies in the sedimentation behavior of larger particles compared to their nanosized counterparts. This sedimentation has been observed in many dialysis-based techniques, including the dialysis bag in combination with *Apparatus 2* or *4* (*56, 59, 61*). A wide variety of dosage forms and performance assays have been developed for subcutaneous and intramuscular routes of administration (46, 57, 60). Under those circumstances, some methods use synthetic diffusion barriers such as hydrogels (*46, 57, 60*) or membranes to mimic the limited fluid volume present at the injection site (*10, 58*). In this context, a fundamental difference in the biopredictive methodologies becomes more apparent.

Hydrogels can also act in a separating capacity when used as a medium. While mimicking the limited availability of liquid and tissue interactions often predicts agglomeration effects or matrix erosion more realistically (*46, 60*), other methods apply shear forces during the separation to discriminate more effectively between drug formulations (*10*). On the one hand, for methods changing the hydrodynamics by means of agitation or a continuous flow set-up (*10, 56*), the in vitro experiments sometimes over discriminate differences between different drug formulations. On the other hand, even slight differences between formulation candidates can be discovered more sensitively and lead to an accelerated dissolution test (*10, 58*).

To mimic the physiological environment more realistically, soft hydrogel-based matrices (*46, 60*) or even muscle tissue were proposed as a suitable model for subcutaneously or intramuscularly injected formulations (*62*). Of note, with the application of biological materials in the assay, these tests often compromise other aspects, such as the duration of the performance assay.

LIMITATIONS OF THE EXISTING PERFORMANCE ASSAYS

In recent years, several release assays for the testing of non-oral complex dosage forms have been developed, including injectable drug products such as emulsions, suspensions, and implants (*52–55*). Analytical challenges include the separation of the dispersed drug from the release medium and the excipients. This becomes even more apparent when biorelevant assays are employed (*53, 56, 59*).

Instruments applied in testing are often designed to reflect the physiology of a specific administration route. For some administration routes, knowledge of the mechanisms of release is very limited and rarely supported by human clinical data, which leads to a certain diversity that contradicts the very aim of harmonized quality control methods (*45, 53*).

Many of the instruments designed for the testing of injectable drug products do not follow well-defined standards, leading to higher variability in release data, impacting reproducibility and variation between different laboratories, as compared to compendial equipment (*56, 59*). The exact dimensions of the instrument, as well as a comprehensive description of the test conditions and sample collection procedures, are required. Depot formulations releasing the drug over many days or months further challenge assay development because of the time constraints in routine quality control. For these products, accelerated conditions play an important role and are discussed in the relevant section of this article. Medium evaporation and changes in the medium composition may occur as a result of the long-term experiment. Biorelevant media sometimes contain proteins that tend to agglomerate under constant shearing in some compendial instruments (*7*). As outlined in previous sections, separation techniques have been covered by the USP *Stimuli* article covering nanomaterial-based drug products (*3*). It discusses criteria for the selection of filter materials, membranes, columns, and other key parameters that need to be optimized during method development.

For some administration routes, poor hydrodynamics and complex distribution processes make the direct correlation with clinical in vivo data more challenging. The right balance between reliable and robust assays and a simulation of physiological complexity has not always been identified for all injectables (*53, 63*). Many of the current efforts in nanomedicine focus on the simulation of tissue targeting to estimate efficacy (*53*). However, these biodistribution processes are often monitored in animal models and may not reflect the human situation (*53*). Although most injectables will interact with the physiological microenvironment in multiple ways, the release is often driven by a selected number of parameters, such as the solubility of the drug in the surrounding liquid or the partitioning of drug molecules (*45, 56*). Simulating this mechanism of release is a key aspect in the development of the optimal set-up and requires a thorough understanding of the physiology of the administration route. For injectables, the medium composition covers a wide range from semisolid or solid hydrogel structures to aqueous buffer systems.

These aspects are discussed in the section on medium composition and selection. Their limitations can only be discussed with reference to the dosage form under investigation. For example, hydrogel assays have been developed to provide a defined diffusion barrier, limiting the availability of liquid at the administration site. This can be of interest for dosage forms and drugs that exhibit a certain solubility, such as proteins or peptides (*45*, *64*). In these cases, the availability of liquid, and not the aqueous solubility of the compound can be responsible for drug transport. These observations have been made for biotechnological drug products injected subcutaneously. For other drugs, including drug microcrystals, the influence of the dissolution rate may play a more dominant role (*10*).

CONCLUSIONS AND RECOMMENDATIONS

Parenteral dosage forms and their associated dissolution tools are evolving as injectable products are increasingly approved. Emerging platforms should transition from the research phase to robust commercializable systems that can become standardized. As this has been successfully achieved in the oral dissolution space, it is being recommended that parenterals follow a similar strategy:

- **General**: Due to the diversity of parenteral dosage forms, it is recommended to sub-divide parenterals into manageable groupings that can be more easily developed into standard tests, perhaps in a similar fashion to the oral Biopharmaceutics Classification System (BCS) (*11*). It is also recommended that a clear rationale is presented for the selection of test conditions, including the model or formula used to determine the release rate. System variability should be characterized to quantify the impact of variability on calculated release kinetics, along with the need to clarify that aspects of a test system are intended to be bio- or clinically relevant.

- **Apparatus and Test Conditions**: Given the range of non-compendial apparatus and equipment used to assess the performance of parenteral products, consideration should be given to commonly used non-compendial apparatus to promote standardization. Leverage those apparatuses that better represent the volume of fluid the dosage form will be exposed to in vivo, even if that requires deviating from traditional dissolution apparatus. Whereas in general, the selection of agitation conditions should promote good dispersion and mixing, intended biorelevant agitation may require lower fluid velocities which could impact particulate dispersion and local sink conditions. The test duration and sampling frequency should be sufficient to characterize release kinetics and, where appropriate, degradation profiles. The potential benefits of alternative or additional characterization methodologies should also be considered to better inform the performance test interpretation, for example, in-situ fiber optics for liposomes and nano-suspensions, and image analysis/sizing methods for poorly soluble active pharmaceutical ingredients (APIs).

- **Accelerated Testing**: It is critical that release mechanisms and the impact of acceleration on these mechanisms are understood, and that the reliability of accelerated conditions to detect altered critical quality attributes of the product is established. Accelerated testing will not occur over a biorelevant timescale. The impact of time-scale compression on in vivo predictability of the accelerated test should be understood. Thus, it is recommended to develop standard linkages from dissolution data to predictive tools, either to translate accelerated dissolution testing to actual drug release predictions or to incorporate the dissolution data into physiologically based pharmacokinetic models.

- **Media**: Standardize representative dissolution media for each route of delivery. Current knowledge of the anatomical and physiological environment should be balanced with existing data on clinical impact. This includes moving away from traditional low viscosity media which has evolved for performance testing relating to the gastrointestinal tract. For some applications, higher viscosity media may be better suited for typical parenteral routes of delivery. In some cases, the use of surfactants may be considered (e.g., non-ionic surfactants to inhibit gel formation in tests for liposomal release, or for wetting in tests assessing microparticle or poorly soluble API performance). However, care should be taken with the use of any surfactant, considering the impact on bio- or clinical relevance and release kinetics. For many parenteral dosage forms, sink conditions cannot be assumed. Estimations of the distribution between the administration site and systemic circulation should be made for each dosage form and drug substance individually. The impact of the local environment on biodegradation is significant for many products, therefore consideration should be

given to incorporating or mimicking these effects in the test environment.

- **Separation Techniques**: The techniques used for the separation of fine particles may involve real-time separation or detection methods as well as sample and separate techniques. They were summarized in another *Stimuli* article published previously (*3*). To understand the key mechanistic or rate-limiting aspects of the drug product design to ensure the separation technique used in the dissolution test is designed appropriately is a key requirement in assay development. When more complex media are used, such as hydrogels, these can more accurately mimic the physiological environment but may also act in a separation capacity and should be evaluated during method development.

This article was written to raise awareness of the diversity and challenges to standardize drug release test methods for injectable and implantable drug products. It is our hope that it will stimulate collaborative and harmonized research to evolve more parenteral test methods to become standards which can be incorporated into future evolutions of <1001>.

Table 1. Performance Tests Currently Employed for Parenteral Products: Limitations, Challenges, and Recommended Considerations for Use.

Dosage Form	Dosage Form (subtype)	Performance Tests in <1001> and Examples of Other Methods in the Literature	Limitations and Challenges	Points to Consider
General	—	—	General methodological challenges: See media, apparatus, and separation section	See *Conclusions and Recommendations* section
Oily Solutions	—	*Apparatus 2* Examples of other methods: *Apparatus 2* with modifications (*65*) Dialysis membrane (*66*)	Medium saturation/sink conditions challenging Membrane compatibility Assembly compatibility Active Pharmaceutical Ingredient (API) stability Burst release -challenging to capture or avoid Membrane size/permeation Partitioning into/between media	Identify effects of donor volume Permeation and distribution coefficients dependent on drug and oil phase composition Consider measuring concentration in donor phase to reflect local in vivo release (e.g., intra-articular), although different in vivo release kinetics (large oil-water interface) and lymphatic clearance should be considered Consider bio-relevance of medium/media relevant to intended route
Suspension (liposome, micro-particle, nano-suspension)	—	*Apparatus 1, 2, 4* Dialysis pouch/reverse dialysis Reduced volume apparatus Filtration Examples of other methods: Accelerated dialysis (e.g., dispersion releaser); *Apparatus 7* (*67*)	Discriminatory capacity of medium Assembly compatibility Difficult to determine dialysis rate Effect of addition of sample to the medium Membrane/filter compatibility Medium evaporation Separation from medium (*3*)	Determination of the dialysis rate (e.g., by addition of a drug solution to the drug formulation) Standardization of data treatment (dialysis rate corrections) 3 recommended considerations for all separation methods for particulates: Selectivity for particle population (size) Analytical sensitivity Sensitivity on time axis (release response–release from dosage form presents as response at what time on profile) Consider bio-relevance of medium/media relevant to intended route
—	—	Filtration	Separation considerations relating to filtration including shear forces, filter pressure, selectivity for different particle populations; Sensitivity to different dissolution rates Medium evaporation Membrane/filter compatibility	3 recommended considerations for all separation methods for particulates: Selectivity for particle population (size) Analytical sensitivity Sensitivity on time axis (release response–release from dosage form presents as response at what time on profile) Verification of filter selection (e.g., by using particle counting methods or qualitative evidence) Determine filter adsorption potential Consider biorelevance of medium/media relevant to intended route

Table 1. Continued.

Dosage Form	Dosage Form (subtype)	Performance Tests in <1001> and Examples of Other Methods in the Literature	Limitations and Challenges	Points to Consider
—	—	Examples of other methods: In vitro perfusion system Cross-flow filtration methods Asymmetric flow field fractionation	Issues specific to asymmetric flow field fractionation (*3*) Strong dilution of the sample Long separation times (>15 min) Incomplete particle recollection Release medium and separation medium are often not identical Eluent composition very limited Particle re-collection must be optimized. Dilution of the sample should not affect release (only slow-releasing systems)	3 recommended considerations for all separation methods for particulates: Selectivity for particle population (size) Analytical sensitivity Sensitivity on time axis (release response–release from dosage form presents as response at what time on profile) Particle re-collection Can all particles be accounted for at the end of the test? Does your method pre-select particles or do you get information from particle size population Consider biorelevance of medium/media relevant to intended route
—	Liposome	*Apparatus 1, 2, 4* Dialysis cell Flow-through with dialysis Other dialysis Centrifugation Ultrafiltration (UF) Examples of other methods: Accelerated dialysis (e.g., dispersion releaser) (*68–69*) Adaptive perfusion system (*69*)	Difficult to determine dialysis rate Agglomeration Gel formation Separation considerations relating to filtration including shear forces, filter pressure, selectivity for different particle populations; Sensitivity to different dissolution rates Challenges relating to accelerated tests may be applicable	3 recommended considerations for all separation methods for particulates: Selectivity for particle population (size) Analytical sensitivity Sensitivity on time axis (release response–release from dosage form presents as response at what time on profile) Determination of the dialysis rate (e.g., by addition of a drug solution to the drug formulation) System hydrodynamics selected for optimal dispersion/reduce agglomeration If using *Apparatus 4*, consider sandwiching or dispersing among glass beads to reduce agglomeration Monitor temperature carefully; consider altering temperature for accelerated testing, but ensure release mechanism unchanged Consider biorelevance of medium/media relevant to intended route
—	Micro-particle	*Apparatus 2, 4* Dialysis methods Incubation jar	May need surfactant for wetting	3 recommended considerations for all separation methods for particulates: Selectivity for particle population (size) Analytical sensitivity Sensitivity on time axis (release response–release from dosage form presents as response at what time on profile) System hydrodynamics selected for optimal dispersion/reduce agglomeration If using *Apparatus 4* consider sandwiching or dispersing among glass beads to reduce agglomeration Consider biorelevance of medium/media relevant to intended route
—	Solid lipid nanoparticle (SLP)	Nanosuspensions *Apparatus 2, 4* Dialysis cell Reduced volume apparatus Filtration	Challenges in generating biorelevant conditions Challenges in replicating tissue targeting Challenges relating to accelerated tests	See liposomes and microparticles section

Table 1. Continued.

Dosage Form	Dosage Form (subtype)	Performance Tests in <1001> and Examples of Other Methods in the Literature	Limitations and Challenges	Points to Consider
—	Poorly soluble API	Any test differentiating effect of particle size Direct measurement of particle size (for example using light scattering methods)	Challenges in generating biorelevant conditions Determination of effective in vivo particle size Challenges with accurate particle characterization for size/shape	Consider biorelevance of medium/media relevant to intended route Solid state and polymorphic form understood and characterized, including relevance to dissolution rate System hydrodynamics selected for optimal dispersion/reduce agglomeration If using *Apparatus 4* consider sandwiching or dispersing among glass beads to reduce agglomeration
Emulsion	—	*Apparatus 2, 4* Dialysis cell Vertical diffusion cell Reduced volume equipment	Lack of consistency in methods Challenges in generating biorelevant conditions	Consider how droplet size affects performance Considerations listed under dialysis and filtration sections above Consider biorelevance of medium/media relevant to intended route Complexity of formulation–how does dissolution set-up (including temperature/ medium composition/volume) impact on formulation stability/integrity including droplet size
Implant	—	*Apparatus 2, 4, 7* Sealed jar Incubation jar	Challenges in generating biorelevant conditions Replication of biodegradation environment Microbial growth Medium evaporation Challenges relating to accelerated tests	Consider use of preservatives and prevention of evaporation as detailed in <1001>, especially considering timeframe of release/release test Consider recommendations relating to accelerated tests
Vascular Stent	—	Reduced volume *Apparatus 2 Apparatus 4 Apparatus 7* with stent holder/ small volumes	Accelerated tests Challenges in generating biorelevant conditions Challenges in replicating in vivo transport/shear forces Analytical challenges with low concentrations Partitioning into/between media Addition of sample to the medium Sample state (e.g., stent open/ closed or before/after sterilization) Suitability of method for QC (e.g., ex vivo perfusion circuit or new replicate for every sample time)	Test conditions in the in vitro test systems should be adapted to some key parameters of the situation in vivo whenever possible There is need for standardization, but there is also still little known about the in vivo conditions There might be need to address both, drug release into the blood stream and diffusion into the vessel wall The vessel-simulating flow-through cell could be a starting point Validation of the in vitro model requires validation set of stents with different release rates which could prove to be an essential problem

Table 1. Continued.

Dosage Form	Dosage Form (subtype)	Performance Tests in <1001> and Examples of Other Methods in the Literature	Limitations and Challenges	Points to Consider
Gels	Two generic subtypes: Those that maintain viscosity pre- and post-injection (viscous oils) Those that increase viscosity (Poloxamer 407, etc.)	*Apparatus 2, 4, 7* Incubation jar for in-situ forming preparations <1001> Examples of other methods: Variety of techniques are used now – most involve a compartment which contains the gel, it could be a dialysis bag or something more elaborate. Generally this is combined with *Apparatus 1, 2, 3*, or *4* or custom. Alternatively, systems having a gel injector such as the SCISSOR kit maybe a good alternative. Or more ex vivo models like Genoskin (*15*) are being developed.	Lack of consistency in methods Specific challenges with gels. Including: Forming the gel prior to testing and how to control the surface area to volume ratio of the gel that likely impacts drug release. In addition, if the gel is contained to maintain its shape there is limited agitation, etc.	Injection of the gel through a representative needle and at a clinically representative rate is critical and should be standardized, as well as a standardize inert, matrix to inject into The injection dynamics can directly impact the gelation and final physical dimensions of the gel including the surface area to volume ratio A simple but consistent approach is best to standardize, such as a dialysis cartridge filled with a standard inert matrix, then placed into an *Apparatus 2* or *4* Limitations such as a high-volume injection versus low volume injection may require different dialysis set-ups Different viscosity gels will require different needle gauge and needle free injectors may not be possible to simulate
Ophthalmic Parenterals	Implants, suspensions, specialized dosage forms such as drug-device combinations	For further information on ophthalmic performance testing, see Ophthalmic Products—Quality Tests <771> and Ophthalmic Products—Performance Tests <1771>	—	Ophthalmic parenterals should be considered on a case-by-case basis due to the specialized and specific nature of these dosage forms If possible, it is advised that the performance test reasonably mimic the method of administration and in vivo conditions, with a view to possibly establishing an in vivo in vitro correlation to predict in vivo performance

CONFLICT OF INTEREST STATEMENT

The authors did not declare any perceived or actual conflicts of interest related to the subject matter of this *Stimuli* article. The views presented in this article do not necessarily reflect those of the organizations for which the authors work. No official support or endorsement by these organizations is intended or should be inferred.

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