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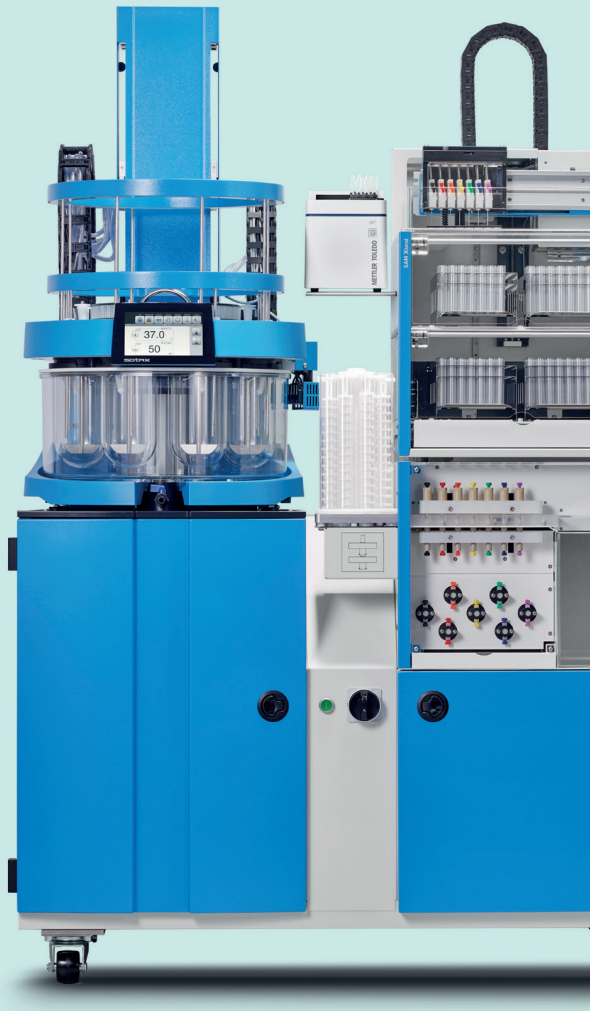

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The **February 2023** issue will include research articles on topical gel dapsone, mannitol, Eudragit, and soft sensor method, as well as a USP *Stimuli* on continuous manufacturing and the Q and A feature.

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Publisher's Information

PURPOSE: *Dissolution Technologies* is a peer-reviewed quarterly publication reporting current information on dissolution testing. It provides an international forum for open exchange of information on various dissolution topics.

CORRESPONDENCE: Send all correspondence to: *Dissolution Technologies*, Inc., 9 Yorkridge Trail, Hockessin, DE 19707. Phone (302) 235-0621, e-mail: vagray@rcn.com. Website: www.dissolutiontech.com

STAFF: Founder, Cynthia Brown; Managing Director, Vivian Gray; Associate Editor, Valerie Clark; Research Editor, Vivian Gray; Communications, Michael Larson; Circulation Manager, Sandra Larson; Layout, Michele Arnold; Publication, Printing, and Distribution Services, Archer Print Group, Sellersville, Pennsylvania.

ISSN 1521-298X

Dissolution Technologies is indexed in:
Science Citation Index Expanded®
Journal Citation Reports®/ Science Edition
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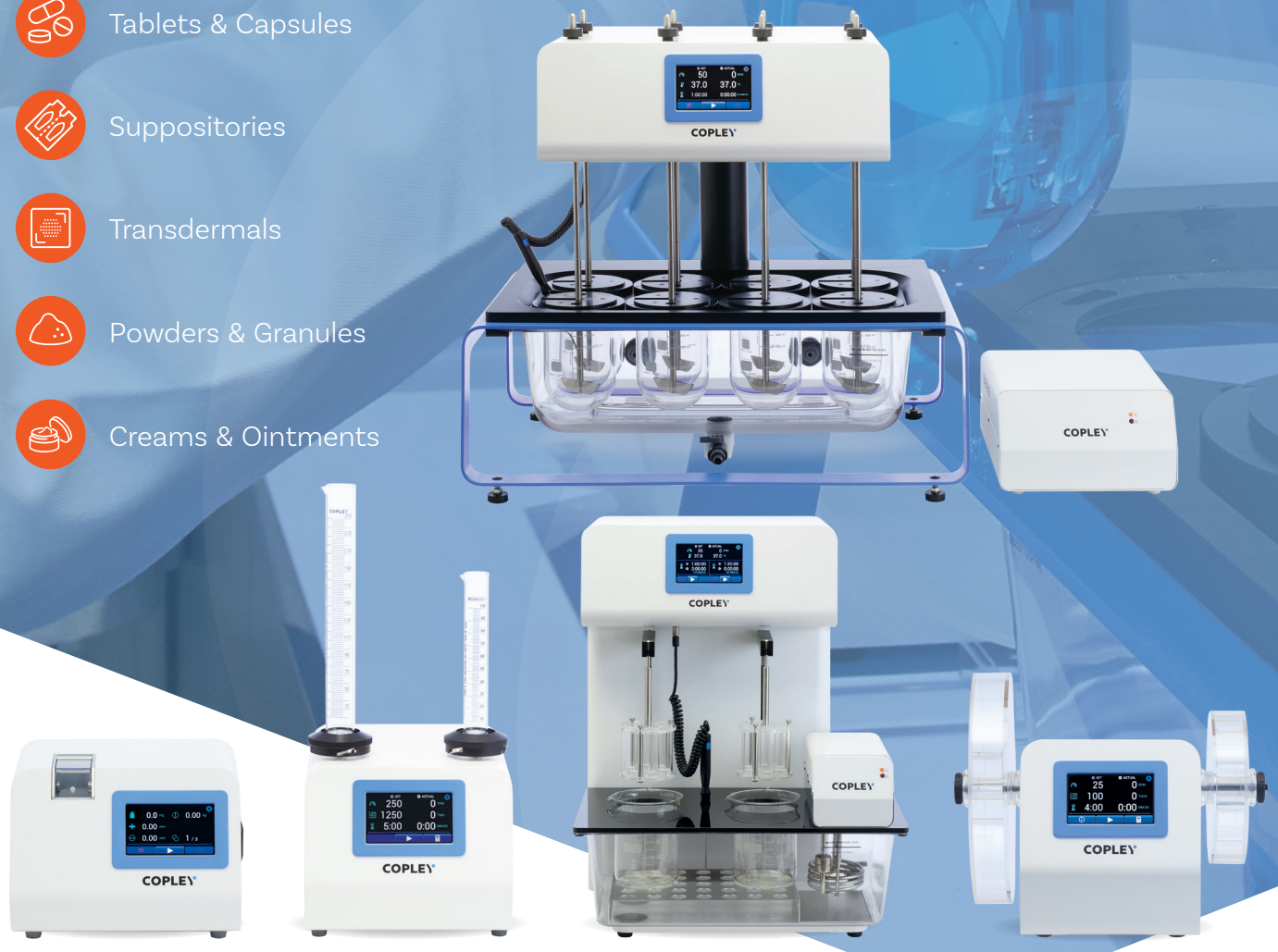

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dx.doi.org/10.14227/DT290422P190

Dissolution Method Troubleshooting: An Industry Perspective

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ABSTRACT

Quality control dissolution testing represents a key product performance test for solid oral dosage forms and is the most likely QC test to result in laboratory investigations because of the relatively complex relationship between the dissolution performance, the drug product properties, and the systems necessary to measure the quality attribute. The Dissolution Working Group of the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ) has pooled our collective knowledge to outline some common ways that dissolution methods can fail. Examples and case studies are given to highlight errors related to equipment, method, materials, measurement, people, and the environment. Best practices for building method understanding and avoiding the exemplified issues are discussed. Case studies highlight the importance of buffer preparation, potential impact of contamination of the dissolution medium, additive-induced degradation, risks in the use of automation, differences between dissolution systems, and the effect of filter selection. Investing in analyst training programs, understanding the capabilities of your equipment portfolio, and using well-designed studies for robustness and ruggedness will reduce dissolution method investigations and improve compliance and productivity during the method lifecycle.

KEYWORDS: Dissolution, troubleshooting, method issues

INTRODUCTION

Dissolution is a key product performance test and a quality specification for almost all solid oral dosage forms. The development of a dissolution method focuses initially on determining conditions that can discriminate for differences in potential product critical material attributes (CMAs) and critical process parameters (CPPs), ideally linking in vitro dissolution to in vivo drug product performance (*1*).

An under-discriminating dissolution method and specification could translate to patient risk through potential release of unsuitable products to market. Equally, an overly discriminating method and specification could lead to patient risk due to the inability to produce product that meets the specification, leading to patients struggling to access their medicine. It is therefore a careful balance when developing a method and selecting a dissolution specification to ultimately assure the quality of product that reaches the patient.

Unfortunately, as a method moves from the development stage into more routine use, either in the clinical phase (release and stability) or later in the commercial phase,

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the sources of variability increase and can lead to scenarios where investigations into the performance of the dissolution method will be needed. This can be mitigated somewhat by performing well-designed studies for robustness and ruggedness during method validation and having well-written methods, a good understanding of the equipment being used, and well-trained staff. However, not all elements or sources of variability can be explored comprehensively during development.

Scenarios that often lead to dissolution method investigations include:

- Out of specification results (OOS);
- Out of trend results (OOT);
- Increased variability in results;
- Increased progression to stage 2 or 3 testing;
- Observations of issues during buffer preparation;
- Unusual visual observations during dissolution;
- Non-comparability during method transfers between laboratories or dissolution equipment; and
- Introduction of automated dissolution equipment.

This review provides commentary on the lessons learned and best practices for dissolution method investigations and troubleshooting, leveraging the group knowledge of the Dissolution Working Group of the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ). In addition, real-life industry case studies are presented to exemplify various ways in which dissolution methods can fail.

DISSOLUTION METHOD INVESTIGATIONS

A dissolution method can be considered as three distinct activities: a procedure for obtaining a sample for analysis, the analysis of the sample, and calculation of the dissolution result from the chemical analysis. As dissolution method issues can occur at any of the three stages, a visualization of the method workflow is a good first step of an investigation.

A fishbone diagram (Fig. 1), also known as an Ishikawa diagram, is a visual presentation of a cause-and-effect system that can help analyze the root cause of the problem and is widely used in the pharmaceutical industry for a variety of applications (*2–4*). It allows brainstorming of all potential causes that might otherwise be ignored. Electronic whiteboard tools have also proven useful to conduct these brainstorming sessions during the COVID-19 pandemic and when conducting investigations across locations within a company or with an outsourcing partner. The arms of the fishbone for dissolution investigations are broken down into six focus areas: equipment, method, materials, measurement, people, and environment. Each of these areas are discussed in the context of a dissolution method investigation.

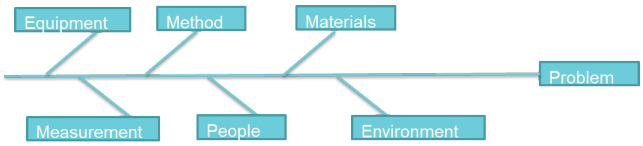


Figure 1. Example of an Ishikawa (fishbone) diagram for dissolution testing.

MATERIALS

Materials to consider in any dissolution investigation primarily fall into three categories: the components used to prepare the dissolution medium, the reference standard, and the drug product that was tested.

Buffer Preparation

For the dissolution medium, simple checks on the reagent weights and that the correct grade of reagent has been used are a good starting point. Common errors observed include hydrated salts such as phosphate not being accounted for correctly. An example is using a dihydrate rather than a monohydrate or anhydrous salt, which has a subsequent impact on buffer concentration. Anhydrous salts, if not stored appropriately, may bind water, which can cause issues in weighing the correct mass of salt needed to prepare the right buffer concentration. Also, monobasic and dibasic salts can be mixed up and adjusted to the correct pH in a different fashion than usual, which gives a different overall composition of medium than if the correct salt was used. This can be avoided by ensuring that a clear order of material addition and the expected pH prior to adjustment is documented and any discrepancies from the expected pH trigger the analyst to pause and check why the pH is outside of the expected value.

For bulk media preparation, adequate mixing must be ensured, which is particularly important when diluting from concentrates to ensure a uniform solution is formed prior to aliquoting. For example, case study 2 demonstrates the need for mixing times of 1 min/L or more for media volumes of 50 L or more in the buffer system. Additionally, if pH is used as confirmation of mixing for larger vessels, then samples should be taken from multiple points at varying depths.

It should also be standard practice to ensure that all reagents have been stored correctly and are within the shelf life assigned to the material. Contamination of the buffer should also be considered, either from a previous dissolution medium using the same equipment that was inadequately rinsed or from microbial contamination. An example of the latter is microbial growth within the helium sparge frit stored in water solution; this issue was resolved by ensuring the helium sparge frit was stored in 50:50 methanol:water between use.

The first case study illustrates a combination effect of incorrect medium preparation and human errors while using a dissolution medium concentrate. The second case study illustrates the effect of incomplete mixing of large volumes of media, as well as the limitations of pH measurement for verification of media preparation quality.

Case Study 1: Buffer Preparation from Concentrate

A dissolution test for a tablet formulation was performed in 500 mL of pH 5.5 acetate buffer using USP apparatus 2 at 75 rpm. For convenience, a 10x concentrate of the buffer was prepared and simply diluted with water to the final buffer concentration prior to every analysis using a media preparation system. A dissolution profile of a development batch following this procedure is presented in Figure 2, showing rapid and robust release. During first clinical batch release, the dashed line profile was observed when applying the previously described method, leading to an OOS result.

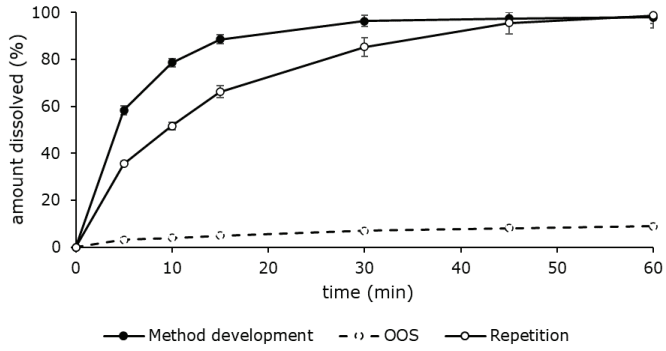


Figure 2. Impact of incorrect buffer medium preparation method on dissolution (case study 1). OOS, out of specification.

During the investigation, it was determined that a final 1x acetate buffer at pH 5.5 was again diluted 1:10, assuming that it was still a 10x concentrate. Thus, the dissolution buffer was prepared at 10-fold lower concentration. The dissolution analysis was repeated with correctly prepared buffer. As depicted in Figure 2, dissolution of the clinical batch met the dissolution acceptance criterion in stage 2 (Q = 80% at 30 minutes). Differences between the development batch and the first clinical batch were attributed to differences in granule particle size distribution, which was analyzed during a subsequent investigation.

In general, the use of buffer concentrates adds a source of variability; however, the time and resource benefit of this procedure is deemed to compensate for this potential error. Further, well-designed control measures like audit trails and documentation checks, even at early development stages, ensure process and product quality.

Case Study 2: Buffer Preparation from Solid Reagents

A dissolution test for a capsule formulation was performed in 900 mL of pH 5.5 citrate-phosphate buffer using USP apparatus 2 at 75 rpm. For convenience, buffer was prepared in large volumes by dissolving solid salts in water in the preparation vessel and agitating until full dissolution was expected. A pH measurement was taken as verification of correct buffer preparation. Fast and robust profiles were typically observed with this dissolution method. However, during primary stability, testing at one time point showed unusually high variability with multiple OOS results. This was observed across multiple capsule strengths, lots, and storage conditions. A systematic investigation of the dissolution data revealed a trend of dissolution behavior with chronological testing time in a particular batch of prepared dissolution media. Figure 3 shows the percent drug release (mean of six replicates) at 45 minutes of various tested batches, plotted as a function of testing order. Each vertical grid line represents a testing day, and separate media preparations are indicated.

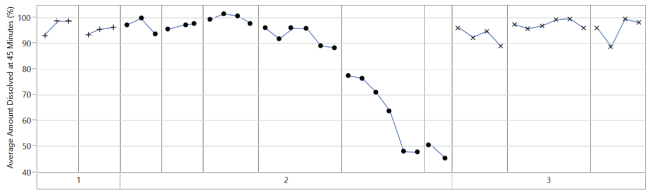


Figure 3. Dissolution release at 45 minutes as a function of media batch and testing order (case study 2).

Further investigation revealed a 150-L preparation of media, agitated for 78 minutes. While neither the agitation time nor the media volume was atypical by themselves, the combination of lower-end mixing time and higher-end volume of media led to a hypothesis that mixing was inadequate and that media composition was inconsistent across its usage. To test this hypothesis, the dissolution samples collected in this batch of media were gathered and tested for pH, conductivity, osmolality, and ion concentrations of sodium, citrate, and phosphate.

Figure 4 shows the dissolution at 45 minutes and media pH and conductivity as functions of testing order. The green

band indicates the expected range of values of pH and conductivity for correctly prepared media. Dissolution performance was directly correlated to the pH of the aliquot of media used. Although pH was in specification for the first half of the media used (resulting in as-expected dissolution), the conductivity of the media was outside of correct range for all but a small fraction of the media around the midpoint of its usage. Similar trends to conductivity were seen for measured osmolality and ion concentrations, although with some offsets. In effect, not one aliquot of the media had the correct composition. Analysis of media volumes and mixing times for this setup's bulk media preparation led to require mixing time of 1 min/L of media or more to ensure adequate mixing for volumes of 50 L or more.

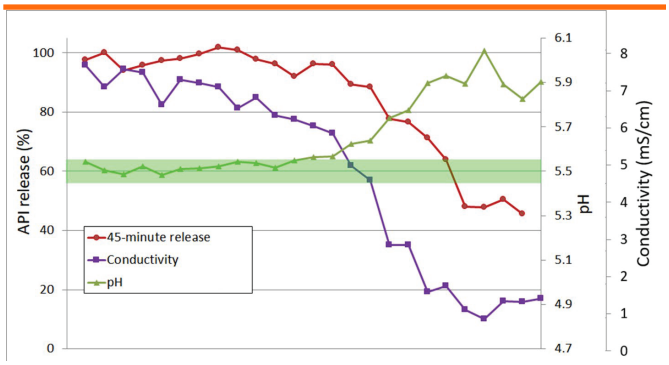


Figure 4. Dissolution at 45 minutes and media pH and conductivity as a function of testing order in inadequately mixed media (case study 2). API, active pharmaceutical ingredient.

This case study indicates that pH measurement is not an adequate indicator of correct media preparation or extent of mixing. If other metrics (e.g., conductivity) are not used, then samples should be taken from multiple points at varying depths of a large vessel if pH is to be used for confirmation of mixing. This also exemplifies the benefits of saving sample solutions until all data are analyzed, trended, and all required investigations are complete.

Surfactant

Surfactants are often a component of the dissolution medium used to achieve sink conditions if the compound exhibits poor solubility. Sodium lauryl sulfate (SLS) is a commonly used surfactant in this context, although it can be a source of dissolution pitfalls such as precipitating in the presence of potassium ions. Different grades of SLS quality can cause interferences due to impurities during the analytical finish of the dissolution test and could impact the solubilization capability of the medium (*5*, *6*). The next two case studies demonstrate the potential unanticipated impacts of surfactant on dissolution, such as surfactant-induced degradation in the samples due to the presence of reactive species (case study 3) and surfactant binding to drug substance, hindering dissolution (case study 4).

Case Study 3: Surfactant-Induced Degradation

Chemical stability of the drug in the dissolution medium can be an important factor to consider during method development. If a drug degrades in the dissolution medium, the amount of drug detected during the dissolution test might be much lower than the actual amount of drug dissolved. Drug degradation is often observed due to chemical instability under specific pH conditions, which should be taken into consideration during medium selection when developing the method. In some cases, impurities in the dissolution medium, which can be introduced by the surfactants, can accelerate degradation of the active drug.

In this case study, compound X, formulated into an immediate-release film-coated tablet, exhibited oxidative degradation, which in some cases caused an apparent decrease in amount dissolved at later timepoints during the dissolution test (Fig. 5).

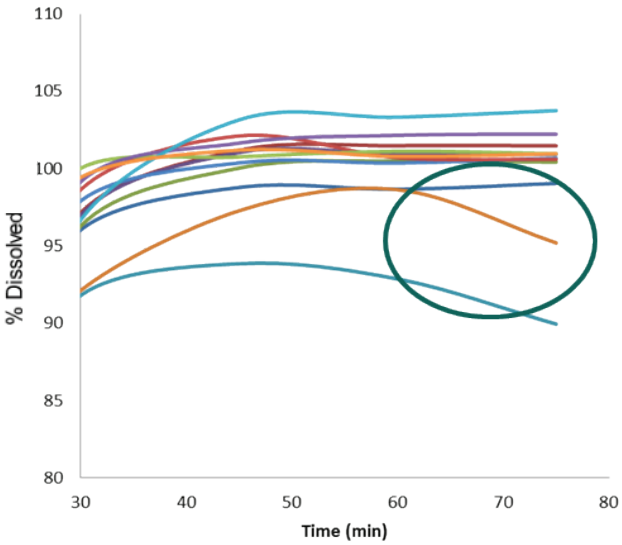


Figure 5. Dissolution curves demonstrating rapid drug degradation in subset of samples from individual vessels (circled) (case study 3).

Even in less extreme cases where degradation did not cause an apparent trend across dissolution timepoints, evaluation of dissolution sample solutions found very limited solution stability of less than 24 hours. Further investigation of the degradation pathway found the growth of two known oxidative degradation products, quantitated by high-performance liquid chromatography (HPLC), in the sample solutions, both of which had been observed to form upon peroxide stress of the active pharmaceutical ingredient (API). This led to the hypothesis that this degradation was due to Fenton-type reactivity with peroxides present in polysorbate

80 (which was included as surfactant in the dissolution medium), catalyzed by iron originating from the film coat of the tablet. The Fenton reaction consists of a conversion of organic peroxides into peroxy and alkoxy radicals via Fe (II)/Fe (III) catalysis (*7*).

Mitigation strategies to reduce the degradation during dissolution focused on both the peroxide and iron components. Polysorbate surfactants are known to undergo oxidative degradation, with buildup of peroxides in the surfactant upon exposure to air (*8*, *9*). The amounts of peroxides were quantitated in several lots of polysorbate 80 obtained from different suppliers and had been opened for varying lengths of time (*10*). Based on these results, the in-use period of the polysorbate 80 was limited to 30 days from opening, and preferred suppliers were identified. Additionally, ethylenediaminetetraacetic acid (EDTA) was added to the dissolution medium to improve sample stability by sequestering the catalytic iron (II) and iron (III) ions, thereby preventing the generation of peroxy and alkoxy radicals. It has also been reported that chelating agents may not suppress the Fenton reaction but instead quench the resulting radicals (*11*). Indeed, this approach was found to significantly reduce oxidative degradation of compound X in dissolution samples, enabling a sample stability of 3 days, with only 0.2% potency loss in that period. Notably, the samples containing EDTA exhibited minimal growth of the characteristic oxidative degradation products compared to samples that did not contain EDTA. The dissolution method was therefore revised to include EDTA in the dissolution medium.

The addition of butylated hydroxytoluene (BHT) to the dissolution medium to quench peroxy and alkoxy radicals was also explored. Lower growth of oxidative degradation products was observed, but a compound X-BHT adduct was formed in sample solutions. BHT was therefore not a viable additive for improved solution stability in this case.

Case Study 4: Surfactant Contamination of Dissolution Media

A dissolution test for a capsule formulation was performed in 900 mL of pH 5.5 citrate-phosphate buffer using USP apparatus 2 at 75 rpm. During dissolution method transfer to a third party, depressed dissolution performance was observed relative to that observed during method development. It was known that the drug substance forms an insoluble complex with SLS at sufficiently large concentrations of SLS. Figure 6 shows dissolution in the method as designed (no SLS) and for a range of SLS concentrations. At 10 ppm SLS, full release cannot be attained.

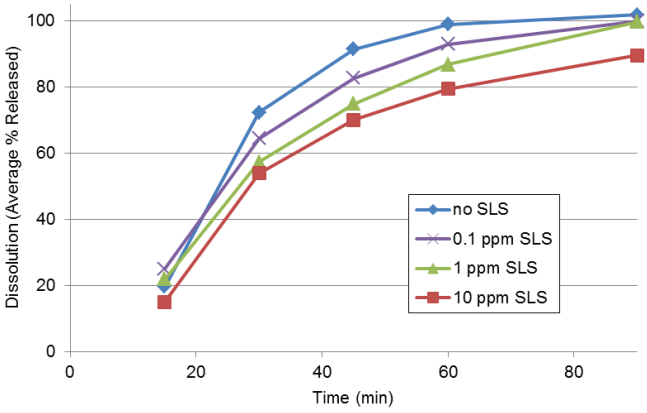


Figure 6. Impact of surfactant contamination of dissolution media (case study 4). SLS, sodium lauryl sulfate.

During the investigation, it was discovered that the media preparation carboy was previously exposed to SLS. Additionally, the dissolution medium leading to unexpectedly low dissolution performance was analyzed with high-resolution liquid chromatography-mass spectrometry (LC-MS) and was shown to contain 0.3 ppm SLS. This level of SLS was consistent with the depressed dissolution performance observed during the method transfer. As a result, new carboys were dedicated to this drug product, ensuring that only this project's dissolution media contacts the surfaces. This practice, as well as full history of equipment and media interactions, can be important for accurate testing of compounds sensitive to trace concentrations of certain impurities.

Enzymes

Another material used in dissolution testing that requires careful consideration is the enzymes used in Tier 2 dissolution experiments when crosslinking of gelatin capsules is observed. For instance, USP general chapter <711> Dissolution specifies that "a quantity of pepsin that results in an activity of not more than 750,000 Units/L" can be added to the dissolution medium during the Tier 2 test (*12*). This means that to correctly calculate the mass of enzyme to add to the medium, the values on the certificate of analysis (CoA) for the USP grade of enzyme need to be considered. Typically for pepsin, the percentage of protein and the units/mg of protein need to be used to correctly calculate the amount needed. Caution should be taken in reading the CoA, as some vendors report percentage of protein and pepsin units/mg protein, whereas others directly report pepsin units/mg product. Alternatively, the activity can be experimentally determined as per the USP procedures. It should also be noted that the USP specification for maximum pepsin activity is given as a concentration. Therefore, in modified Tier 2 methodology where enzyme is added to

a lower volume of buffer prior to addition of surfactant, the amount of enzyme added should be appropriately calculated for the smaller volume.

Reference Standard

The reference standard should be confirmed for identity and relevant purity, taking additional care for potency conversion when the standard is a different salt or co-crystal form from the drug substance to be analyzed in dissolution. UV analytical finish is commonly used as the dissolution detection method. Thus, the UV purity value of the reference standard is often different from that used in a chromatographic analysis due to the accounting of organic impurities in the final value.

Sample

Finally, the dissolution sample itself is subject to variability and error and thus should be examined to ensure its correctness, appropriate labeling, proper laboratory storage, and correct packaging. Often, dissolution method investigations conclude that no issue with method or analysis has been identified, which triggers further investigation into the manufacture of the product. This level of investigation is outside the scope of this paper. However, it can often be useful to have a control or reference sample available for use in method investigations where the expected performance of a particular sample is well understood, as this can help determine if the problem is related to the method or to the individual batch being tested.

EQUIPMENT

The single biggest cause of method issues is the dissolution equipment. This can be due to methods being run on essentially the same apparatus but with analysts unaware of some fundamental differences that exist between manufacturers, bath models, automation approaches, and/or software.

Simple initial checks during an investigation on the equipment can be as straightforward as checking if anything is different from the previous experiments and visual review of equipment maintenance. A check of the instrument logbook, the run report, and any error logs from the experiment can often identify anything unusual in the system before or during the run. The qualification status of the bath, ensuring all pre-run checks, e.g., temperature and paddle height, should be verified. An example was observed where an analyst failed to conduct the correct pre-run checks and failed to observe that the paddle of one vessel had slipped below the 25-mm height and was impinging upon the sinker.

Paddle material and condition should be confirmed, as there have been observations of degradation of metal surfaces with acidic medium, leading to metal-catalyzed degradation of drug substance. This can also be an issue with sampling cannula and autosampler needles. Hence, ensuring that the equipment is well maintained and free of any surface rust are key steps to ensuring consistent results. Polytetrafluoroethylene (PTFE)-coated paddles can be used to overcome this issue; however, care is needed that the coating does not become scratched, as the scratches can lead to areas of degradation or sites for nucleation during experiments where supersaturation occurs.

If baskets (USP apparatus 1) are used, then a check must be made that the correct mesh size has been used and that the condition of the baskets are acceptable, as they are often prone to becoming misshapen due to poor handling. To prevent this, a tool is available for inserting and removing baskets without deforming the mesh.

Degassing

If degassing is critical to method performance, then degassing equipment should be checked to ensure it is providing medium of sufficient quality. This can be done by an external check of the medium with a dissolved oxygen meter to ensure a concentration below 6 mg/L at 37 °C (*13*). Examples of degassing failures are slower dissolution due to the presence of bubbles on the surface of an erodible tablet, causing reduced tablet contact with the medium, as well as reduced medium flow through a basket mesh due to occlusion of the mesh with bubbles. Faster dissolution due to inadequate degassing has also been observed when air bubbles increased the buoyancy of particles and caused a decrease in coning, leading to more dispersed solids throughout the vessel.

UV spectrophotometer

The UV spectrophotometer is an unlikely source for issues if it passes instrument self-tests; however, it is important to confirm correct method settings for the UV wavelength. If a single vessel OOT issue is found using online UV with a cell changer, then it should be checked that the correct pathlength cuvette has been attached to that vessel line. It is also worth checking that all fittings to the cuvettes are secure, as loose fittings can lead to introduction of air into the lines or failure to pull the correct sample volume through the cuvette, which can cause abnormal readings that impact the dissolution profile.

Chromatography (e.g., HPLC) equipment issues are outside the scope of this article. Many books and guides have been devoted to troubleshooting of chromatographic methods (*14*, *15*).

Automation

Automation of dissolution methods and transfer between automated systems is often a source of problems. This can come from a lack of understanding by the analyst of how the system collects a sample. Issues with incorrect selection or definition of autosampler parameters such as prime volumes, purge volumes, pump flow rates, and system tubing volumes can lead to problems. These settings reside not only in the individual method settings but also as part of the configuration file of the system, and volumes are different depending on whether you are collecting into vials or are conducting online UV; the volume will change if syringe filters are used, for instance. Incorrect settings can cause samples to not be taken at the required time due to the autosampler being unable to complete all activities before the next timepoint, or insufficient priming and purging can leave the previous timepoint in the sampling lines, which then dilutes the next timepoint, giving a lower-than-expected result. The impact of autosampler settings is demonstrated in the fifth case study.

Case Study 5: Autosampler Settings

Dissolution testing of an immediate-release tablet formulation was performed on USP apparatus 2 for 12-month stability samples stored at 25 °C/60% relative humidity (RH) and 30 °C/75% RH. The 30 °C/75% RH samples were run on a DISTEK Evolution 6100 bath with a DISTEK Evolution 4300 Autosampler, while the 25 °C/60% RH condition samples were run on a DISTEK Symphony 7100, also with a 4300 Autosampler. The dissolution profile of the 30 °C/75% RH samples was slower than the 25 °C/60% RH samples. The difference in percent drug dissolved was nearly 40% at 5 min and about 10% at 60 min. The difference was not previously seen at earlier stability time points. The 12-month 30 °C/75% RH profile was also OOT compared with profiles from earlier stability time points.

During the preliminary lab investigation, it was discovered that the two autosamplers, although of the same model, had different method settings. The autosampler that was used to run the 30 °C/75% RH samples had a pump flow rate of 10 mL/min with a collection offset volume of 2.0 mL, while the autosampler used to run the 25 °C/60% RH samples had a flow rate of 15 mL/min with an offset volume of 3.5 mL. The offset volume is defined as the discarded medium volume before sample collection. It was hypothesized that the differences in dissolution profiles were caused by the differences in autosampler settings.

The 12-month 30 °C/75% RH tablets were run again with the autosampler method setting changed to 15 mL/min flow rate and 3.5 mL offset volume. Figure 7 shows the comparison of two dissolution profiles of 30 °C/75% RH tablets from two different autosampler method settings. The new profile obtained at 15 mL/min flow rate and 3.5 mL offset volume was faster than the profile previously obtained at 10 mL/min flow rate and 2.0 mL offset volume. With the changed autosampler method setting, the profile of the 12-month 30 °C/75% RH samples matched the 25 °C/60% RH samples as well as the historical trend from previous stability time points (using the same autosampler settings).

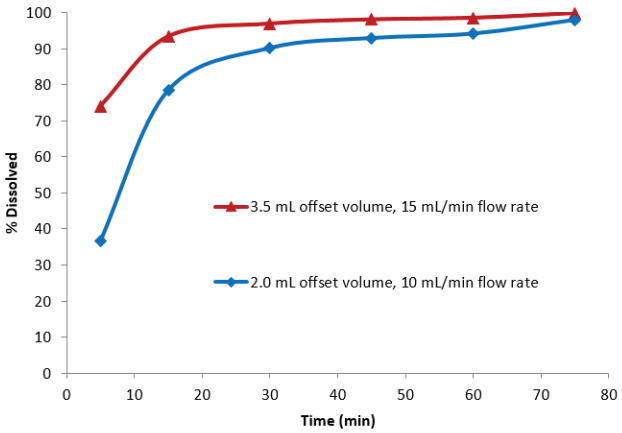


Figure 7. Impact of autosampler method settings on dissolution (case study 5).

To investigate which parameter was more critical, the flow rate or the offset volume, the 30 °C/75%RH samples were run again with an autosampler setting at 10 mL/min flow rate and 3.5 mL offset volume. No significant difference in dissolution profile was observed compared with the profile previously obtained with 15 mL/min flow and 3.5 mL offset volume, indicating that the low offset volume (2.0 mL) was the root cause of the seemingly slower dissolution profile from the initial run. The lower offset volume was insufficient to clear out the sample left in the tubing from the previous sampling timepoint.

Lastly to further confirm the finding, a pre-dissolved drug solution was prepared and used for two runs with different autosampler settings (2.0 vs. 3.5 mL offset volumes, 15 mL/min flow rate for both). Sampling needles for each vessel were placed in water for the 5 min time point, then into vessels containing the pre-dissolved solution for the next time point at 15 min. With the 3.5 mL offset volume, the result showed nearly 100% dissolved at 15 min, consistent with the pre-dissolved concentration. With the 2.0-mL offset, the result was less than 65% dissolved (recovered), indicating a significant dilution effect by the water left over in the tubing. This observation

confirmed that the 3.5-mL offset volume was sufficient for flushing out the previous sample, while the 2.0 mL was not. This case study demonstrated the importance of understanding how the autosampler functions and using sufficient offset volume to displace the previous sample in the tubing and to ensure the sample is representative of the actual sampling point.

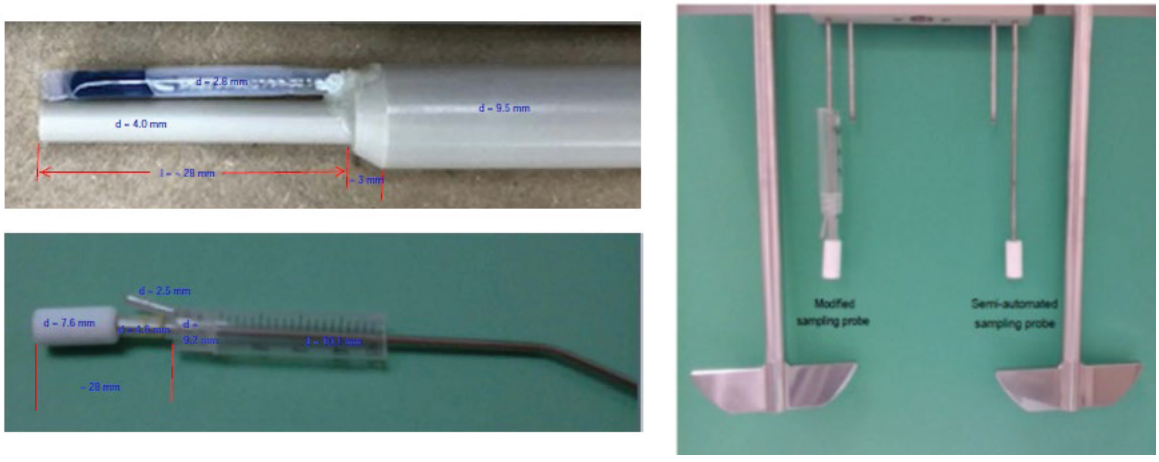
Other issues with automation observed by the group include 0% dissolved in one vessel, followed by 200% in the next run due to a tablet getting stuck in the sample magazine. This issue can be caused (or exacerbated) by tablet geometry, and it may be necessary to ensure consistent alignment of the tablet's smallest dimension with the aperture in the tablet dispensing mechanism of the dissolution system.

Another commonly observed issue for fully automated systems with valves in the bottom of the hemisphere is exacerbated coning for formulations prone to it, which is due the overall "flatter" vessel bottom compared to conventional design. The next case study focuses on the transfer of a method between automated equipment and how differences in equipment design can lead to hydrodynamic differences. These hydrodynamic differences can lead to large impacts in the release profile for products with a sensitivity to hydrodynamics.

Case Study 6: Automated System Differences

Differences in dissolution profiles were noted between different instruments (Varian VK 7000 semi-automated system and Sotax AT MD fully automated system) when using the same method with the same batch of a solid oral drug product. The method was USP apparatus 2 at 75 rpm, pH 3.5 buffer. A coning effect was observed in the dissolution profile from one instrument but not in the dissolution profile from the other instrument.

Upon inspection of the two instruments, it was noted



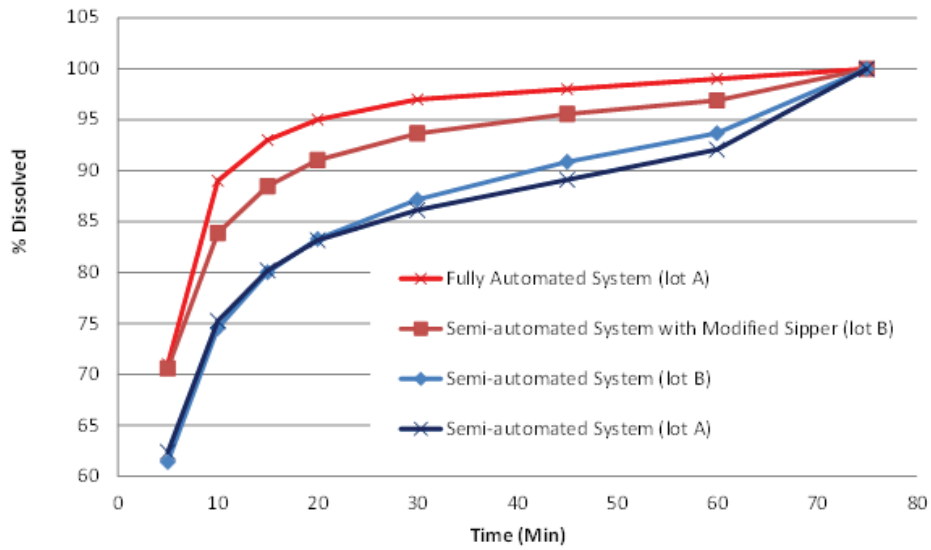


Figure 8. Impact of automated system differences on dissolution (case study 6). Top: Automated sampling probe, semi-automated sampling probe, and probe modified to mimic the fully automated sampling probe. Bottom: Dissolution profiles using USP 2, pH 3.5, 75 rpm through 60 m followed by an infinity spin for 15 min.

that the sampling probe for the fully automated system had a larger diameter than that for the semi-automated system. The difference in size of the sampling probes could potentially lead to differences in the hydrodynamics within the vessel and cause differences in sample deposition or coning between the two systems.

Three sampling probes that mimicked the dimensions of the sampling probe for the fully automated system were constructed to replace three out of the six sampling probes on the semi-automated system (Fig. 8A).

A dissolution run was performed to compare dissolution profiles and coning behavior on this system using the two types of probes. At the completion of this dissolution run, the location of the two types of sampling probes were switched and a second dissolution run was performed to eliminate any potential bias arising from vessel position. These mean dissolution profiles (labelled lot B) are shown in Figure 8B along with previously obtained dissolution profiles for the semi-automated system (labelled lot A), with their respective sampling probes.

The use of the modified sampling probes in the semi-automated system altered the dissolution profile. There was less of a coning effect observed at 60 min, and the profile looks more similar to the profile obtained using the fully automated system.

The method was updated to use apex vessels to minimize coning effects, eliminate the sensitivity to sipper dimensions, and achieve reproducible dissolution profiles between instruments (*16*).

Similar to this example, the following case study also focuses on automation and how small differences in the vessel design and setup can influence dissolution.

Case Study 7: Differences Between Manual and Automated Systems

Manual sampling is usually used in the reference method during development, as this method can easily be transferred to other sites due to the standardization in USP instruments. Dissolution automation can be introduced to increase throughput if similar results to the manual method are obtained.

In this example, a fully automated system (Sotax AT70 Smart) was used during early development. A change in the formulation process resulted in an altered disintegration behavior of the tablets and made it necessary to re-evaluate the comparability between the manual and automated systems. For the profile comparisons, three different Sotax instruments were used: an AT7 smart as a standalone instrument for manual sampling with offline UV spectrophotometer

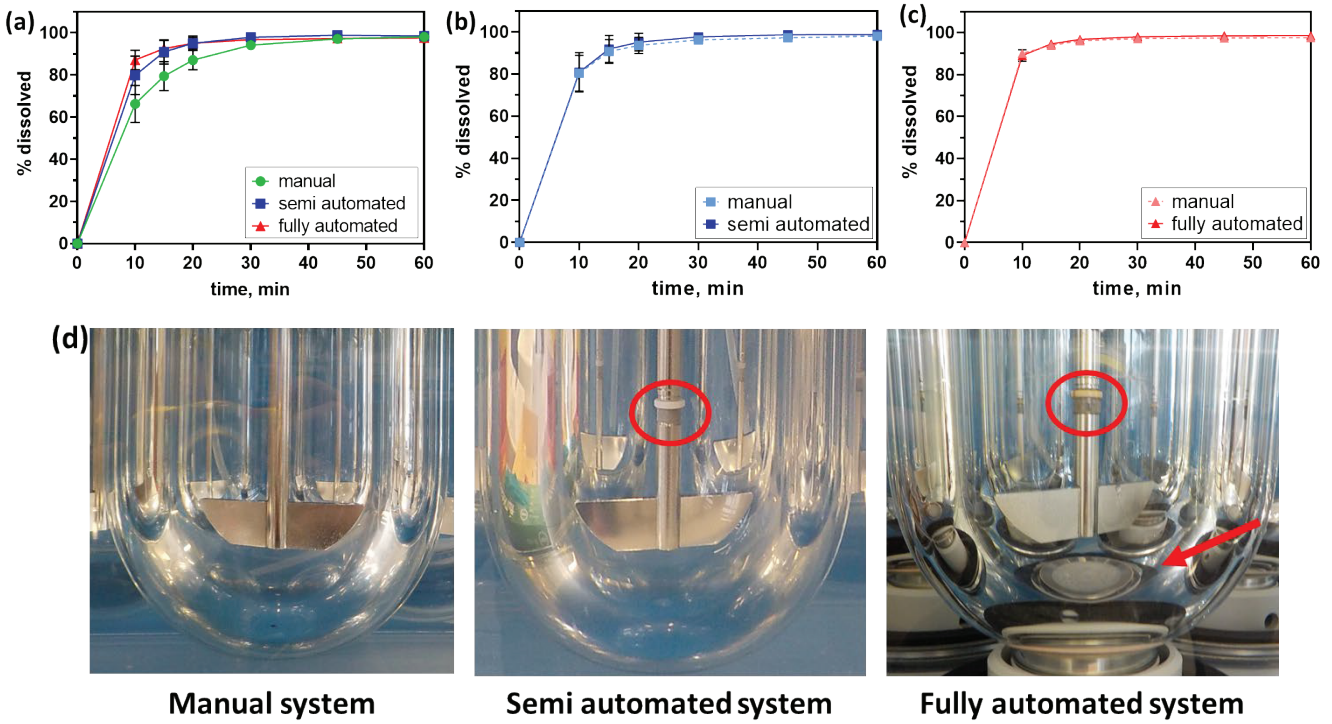


Figure 9. (a) Dissolution profiles using manual, semi-automated, and fully automated setups (case study 7). (b) Comparison between parallel manual and semi-automated sampling. (c) Comparison between parallel manual and fully automated sampling. (d) Vessel setup in each system with marked hollow shaft (red circle) and bottom valve (red arrow).

measurements, an AT7 smart coupled with an online UV spectrophotometer for semi-automated measurements, and an AT70 smart with an online UV spectrophotometer for fully automated measurements. For dissolution, the paddle apparatus was used with 900 mL of pH 4.5 buffer with 0.2% SLS and 75 rpm.

The manually drawn samples resulted in the slowest and non-similar release profile compared to both automated systems (Fig. 9A), with the AT70 Smart measuring the fastest dissolution rate. To investigate influences in the semi and fully automated system during online UV measurements such as tubing and pump volume, manual samples were drawn alongside dissolution runs in both systems. Manual sampling with parallel offline measurements resulted in similar dissolution profiles compared to automated sampling and measurements (Fig. 9B and 9C). Hence, the cause for the dissimilar dissolution profiles had to be the result of something in the vessel itself. In contrast to the AT7 Smart bath, which uses retractable cannulas during manual sampling, samples in both automated systems are drawn through a hollow shaft sampling port. This sampling port is a small mesh insert (Fig. 9D), resulting in a partially non-smooth surface within the paddle shaft. In addition, the bottom valve in the AT70 Smart (Fig. 9D) is an insert into the normally smooth glass bottom of the dissolution vessel. Therefore, both hollow shaft and bottom valve can influence the fluid dynamics within the dissolution vessel and create local differences in fluid flow field. In this case study, the tablets were extremely sensitive to changes in fluid dynamics within the vessel, resulting in increased disintegration and dissolution. This made it impossible to establish an automated dissolution method using the Sotax baths.

METHOD

During any investigation, a check of method parameters should be performed against the approved method. These include paddle speed, vessel temperature, medium, reference standard preparation, sampling, and timepoints. Examples exist where issues have been identified with methods being run at 50 rpm instead of 75 rpm; medium temperature dropping outside of the range of 37 ± 0.5 °C due to close proximity of sampling timepoints and having medium cool in the lines during sampling on systems that retain volume in tubing between timepoints; reference standard not fully solubilized during preparation and leading to lower than calculated standard concentration; and sampling using in situ sampling probes that have not been validated with the method. Problems have also been observed with methods not conforming to pharmacopeia limits for sampling times due to (with manual dissolution testing) analysts adding drug products to all vessels almost simultaneously. This situation results in the later vessels being sampled outside the 2% window because the analyst cannot sample and filter quickly enough. Also, failure to stop paddles prior to dropping a tablet into a vessel has resulted in cases where the tablet is beaten by the moving paddle as it sinks into the vessel, resulting in faster dissolution. Sampling outside the pharmacopeia zone of halfway between the top of the paddle and the level of the medium can also occur when moving between 500 and 900 or 1000 mL volumes if one does not correctly adjust the sampling manifold.

Filters

The dissolution filter can be a culprit in dissolution issues, with missing or only partial filter validation completed (*17*). Filter validation should ensure that the discard volume is established correctly and is performed at the lowest concentration expected in the dissolution profile (e.g., the first timepoint at the lowest strength). An example has been observed when a filter was changed on a commercial product and discard volume selection was only performed at the nominal 100% dissolved concentration of the middle strength. The selected discard volume was later shown to be insufficient to properly saturate the filter when testing the lower strength and led to artificially lower dissolution results, which eventually led to OOS results.

The second element of the filter validation that must be completed is a check of filter efficiency. This can be conducted by sampling at a timepoint where undissolved material will be present and filtering using the discard volume. The filtrate should then be split, with one portion analyzed immediately and the second portion sonicated or subjected to another alternative solubilization method for a period of time before analyzing. If the filter is inefficient in stopping undissolved drug, then the second sample will give a higher concentration than the original sample. It is particularly important to eliminate this issue for LC methods where the sample could reside on an autosampler for hours and where organic solvent is used in the mobile phase, both of which may lead to dissolution of drug particles. These particles would then dissolve in a smaller sample volume than in the vessel, having a disproportionate impact on sample concentration. Inefficient filtering can also cause issues in UV methods due to the undissolved particles (of drug or excipient) leading to light scattering effects that elevate the baseline and require a correction technique to be applied to compensate for them. Ideally, the filter should be efficient to stop all particles passing into the sample.

Usually, a 0.45-µm membrane is more than sufficient to filter out most drugs and excipients, although many automated systems can now handle the back pressure from a 0.22-µm membrane filter.

The last check for filter validation is an evaluation for leachables, performed by filtering a blank solution of dissolution medium and analyzing the filtrate for interfering species. Most reputable filters do not have issues with common dissolution media, but examples have been observed in some low-quality filter membrane providers.

Case Study 8: Early Spikes in Dissolution Profiles

A dissolution study was performed in which a higher percentage of drug was dissolved at early time points than at the subsequent time points (Fig. 10).

In this case, undissolved material was sampled, which was collected on the filter surface and dissolved during the filtration process, resulting in higher measured concentrations. The significance of this effect depends on the dissolution behavior of the undissolved particles on the filter surface, sampling volume, and pressure applied during sampling.

The solution to remove this issue was three-fold:

1. Use a pre-filter attached to the top of the sampling probe.
2. Sample lower volumes to ensure minimum discard volume is still achieved (with the consequence of changing the assay method from UV -spectrophotometry to HPLC analysis).
3. Carefully describe the sampling procedure in the written methods.

Another potential challenge is the filtration of sample solutions with the same filter across all time points for a dissolution profile determination. This might happen to avoid the cost-intensive use of filters at each individual time point. A carry-over of undissolved material on the filter surface can happen in these cases, with the consequence of their dissolution at the next time point causing a higher concentration in the sample and artificially higher measured dissolution. This is also critical from the perspective of false positive results shifting a failing dissolution performance at the specification time point into acceptance. Any multiple use of filters therefore needs careful evaluation regarding those carry-over risks.

In addition to choosing the right filter and establishing a protocol to allow reproducible results, the geometry of the filter casing can also have an impact on sampling.

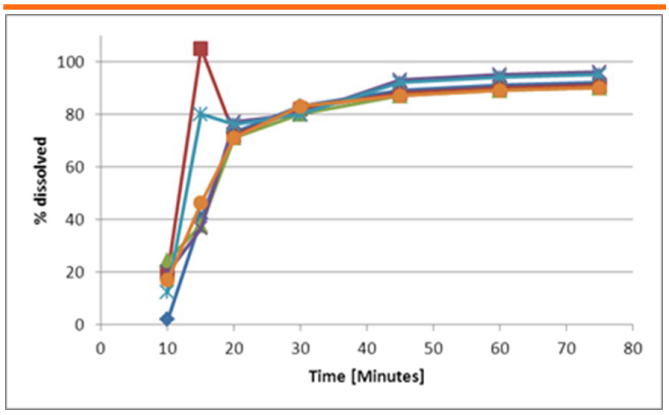


Figure 10. Individual vessel profiles highlighting high percent dissolved values at early timepoints resulting from sampling of undissolved material (case study 8).

Case Study 9: Filter Casings

An increase in dosage strength made it necessary to include surfactant in the dissolution method (paddle apparatus, pH 4.5 buffer with 0.3% SDS, 75 rpm). The dissolution was usually performed on a Sotax AT7 smart system with an automated sampling device (ASD) unit. During sampling with the ASD unit, a syringe plunger pushes air through the syringe filter and cannula into the dissolution vessel to remove potentially stuck particles. Then the ASD pre-rinses the filter by drawing a sample and pushing it back prior to taking the sample, which is subsequently transferred into an HPLC vial. The addition of SLS to the dissolution medium in combination with a 1-µm Pall Acrodisc filter (Fig. 11B) resulted in foaming and incomplete to no sampling (Fig. 11A). Using a 1-µm Pall Acrodisc PSF filter, which is made from the same material as the original filter but has a smaller, differently formed casing (Fig. 11B) eliminated the foaming issue (Fig. 11A). Although this example may seem especially sensitive to sampling with the ASD setup, it illustrates the importance of not just the filter material and pore size but also casing geometry.

Sinkers

Sinkers can result in method issues. It is important during development to evaluate the impact of sinker design on dissolution method performance. An example exists for an oral controlled-release product where the release from the formulation depended heavily on the initial hydration of a polymer. During routine dissolution testing, seemingly random faster releasing tablets were observed and triggered an investigation. The root cause was determined to be related to sinkers: a single set of six non-compliant five-coil Japanese-style sinkers was mixed into a box of 36 compliant seven-coil sinkers. The reduction in the number of coils gave a more rapid erosion of the formulation prior to the full hydration of the polymer. This would suggest that laboratories should control sets

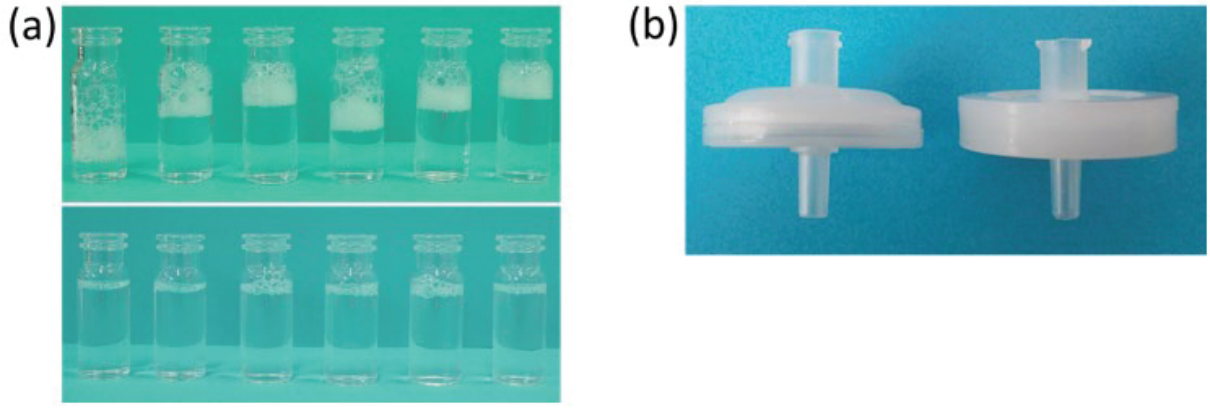


Figure 11. Impact of filter casing geometry on sampling (case study 9). (a) Top row: incomplete sampling due to foaming; bottom row: changing filter type resulted in consistent sampling with almost no foaming. (b) Different casings for the same syringe filter. The square edge Acrodisc filter (right) resulted in foaming shown in A (top row). Smaller filter casing (left) resulted in no foaming, shown in A (bottom row).

of sinkers carefully and adopt a library-type system to ensure sets are labelled and documented before being introduced into the laboratory. It is also important before implementing any switch in sinker design for an approved product to conduct a full risk assessment to ensure equivalence with historical data.

Other commonly seen issues are related to methods where the drug substance is supersaturated relative to its equilibrium concentration, as illustrated by the next case study.

Case Study 10: Post-Sampling Precipitation

Comparative dissolution testing of a weakly basic development drug with pH-dependent solubility was conducted at a contract manufacturing organization (CMO) in 900 mL simulated gastric fluid without enzymes (SGFsp), acetate buffer pH 4.5, and simulated intestinal fluid without enzymes (SIFsp) pH 6.8 using the paddle apparatus at 50 rpm (+ infinity spin 200 rpm after 60 min). Dissolution was fast, robust, and complete in SGFsp, but high variability and unexpectedly high dissolution values (relative to low solubility) were observed at pH 4.5 and SIFsp pH 6.8 (Fig. 12A).

Because solubility at pH 4.5 and SIFsp pH 6.8 limits the dissolution process and because dissolution samples at the CMO were not diluted prior to HPLC analytics, the hypothesis of drug supersaturation and precipitation during/after sampling was evaluated. Repetition of CMO experiments within an internal company laboratory without dilution confirmed high variability and unexpected high dissolution values.

In contrast, the introduction of a dilution step (1:1 with 0.1 N hydrochloric acid) after filtration and prior to HPLC analytics yielded substantially lower (as expected) and more robust/less variable dissolution results for pH 4.5 and SIFsp pH 6.8, as indicated in Figure 12B. Consequently, it can be assumed that during HPLC analytics, precipitated drug particles are most likely being withdrawn from HPLC vials and injected into the HPLC system. In turn, injection of precipitated particles that were diluted with mobile phase during HPLC runs caused high variability and overly high "local" drug concentrations on the HPLC column.

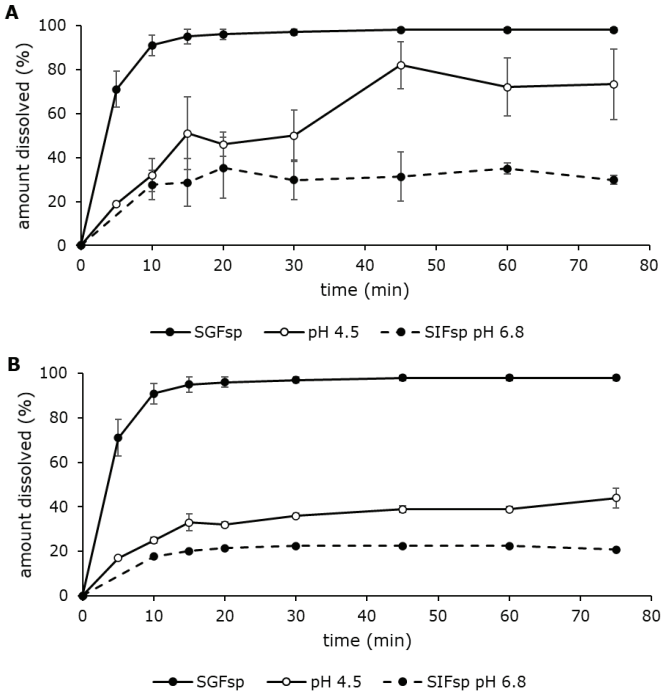


Figure 12. Comparison of dissolution in SGFsp, pH 4.5, and SIFsp pH 6.8 (A) without and (B) with dilution of samples that are prone to post-sampling precipitation (case study 10). SGFsp: simulated gastric fluid without pepsin; SIFsp: simulated intestinal fluid without pancreatin.

ENVIRONMENT AND PEOPLE

Dissolution problems, like all lab-based problems, can result from conducting the tests in poorly thought-out

locations or by individuals with insufficient training. An example of insufficient level of training was seen where a pronounced positive bias was observed from vessel 1 to 6 on manually sampled dissolution. The issue was caused by the analyst dropping the tablets with a 1-minute stagger with the paddles not turning for the entire time; stirring commenced after the tablet in vessel 6 was dropped, and then sampling at 15 minutes with a further 1-minute stagger. The end result of this procedure was that the tablet in vessel 1 experienced 5 minutes of stagnant "soaking" and then 15 minutes of paddles turning, whereas vessel 6 experienced 20 minutes of paddle turning, with other vessels in-between. This was identified as a lab training issue and was resolved by retraining analysts on the dissolution technique at the affected laboratory as well as introducing clearer operating procedures for conducting manual dissolutions (i.e., only stop the paddles long enough for the tablet to sink and then turn them on again for the stagger time).

Dissolution is a technique where visual observation can be of great importance. The first question during an investigation from the expert will normally be, "what did it look like in the vessel?" Having analysts who are well trained in making observations during dissolution runs and taking photographs or videos using mobile phones or other lab recording devices when observing a potential issue can often prove invaluable in finding the root cause. Alternatively, an instrument setup with properly placed cameras and matter-of-course video recording of all dissolution tests can be especially helpful during development and reduce the burden on analysts to note outlier activity, perform observations, and/or record evidence while adhering to the sampling timeframe requirements. Observations of coning, "dancing," floating, pellicle formation, rupture points during capsule dissolution, excessive air bubbles, foaming, or material sticking to paddles/vessels are invaluable at determining if there is any visually observable reason for the aberrant dissolution performance. It is therefore good practice to train analysts to routinely document visual observations when conducting dissolution tests.

An important step in any dissolution investigation is an analyst interview or method walkthrough (sometimes known as a Gemba walk) (*18*). Many breakthroughs in investigations are made by observing the test being performed in the laboratory rather than assuming the test is being performed as per the manager or specialist expectations. In one instance, a sudden change in method performance was observed, and only during the method walkthrough did the dissolution specialist discover that another piece of equipment, which was installed by a different group, caused excessive vibration through the lab bench, affecting the dissolution test.

The final component of environment and people is data integrity and verification. When aberrant results are observed, data should be checked thoroughly by a second scientist and the error confirmed, making sure there is no simple explanation such as a transcription or calculation error. All aberrant dissolution data should be checked as per the laboratory second scientist review process before any investigation is commenced.

MEASUREMENTS

The final area on the fishbone diagram is the measurement of drug concentration in the standard and sample solutions. Method system suitability criteria should be checked and trended to ensure operation within expected ranges. Unusually high or low standard responses may point to a problem with the weighing or dissolution of reference standard, or incorrect flask size, UV cuvette pathlength, or wavelength.

If using chromatography, it is prudent to check mobile phases to ensure they have been prepared correctly, are within shelf life, have the correct pH, and are installed on the correct mobile phase lines. Equally, a check should be made on the chromatographic column to ensure the correct phase, dimensions, and particle size have been selected.

The dissolution calculations themselves can be the root of the problem if the method is not explicit in how to perform them or if it is early in development and the method is not fully defined and validated. Problems have arisen by incorrect or inconsistent use of calculations for the percent dissolved value. These normally arise from failures to account for changes in volume during the run due to sampling and needle rinses. This can easily be avoided by the use of a validated tool and/or an off-the-shelf calculation tool to process the data. Consistently low or high results across all vessels are often related to calculation issues or dilution factor issues.

The use of individual vessel corrections for tablet weight, assay, or normalization to the infinity spin timepoint should be done with care and should be clearly labelled as data that have been corrected, so as not to draw incorrect conclusions when making comparisons to non-corrected historical data.

Finally, it is important to ensure that all analyses were conducted within the stability window for both sample

and standard solutions and that all were stored correctly within the laboratory (e.g., protected from light if required), as failure to store the samples as per the validation would potentially invalidate any data.

CONCLUSION

Dissolution methods are multivariate. To ensure the results are reflective of the true product performance and to prevent incorrect conclusions about product performance, it is imperative that the appropriate controls are introduced in the dissolution method. A firm understanding of potential issues with the method, equipment, materials, measurements, people, and environment is needed to ensure robust and reproducible dissolution performance. Minimizing variability in operational factors will allow enhanced product understanding and avoid costly investigations later in the product lifecycle. Investing in analyst training programs, understanding of the capabilities of your equipment portfolio, introducing quality controls such as audit trails and documentation checks, and prioritizing well-designed studies for robustness and ruggedness should lead to fewer dissolution method investigations.

CONFLICT OF INTEREST

The authors disclosed no conflicts of interest related to this article.

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STIMULI TO THE REVISION PROCESS

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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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dx.doi.org/10.14227/DT290422P220

Review: Application of Bioequivalence Testing of Medicines in Peru

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ABSTRACT

This is a review of the current status of drug bioequivalence studies in Peru. A bibliographic search was conducted in PubMed (Medline database) for bioequivalence studies in Peru. Generic drugs constitute the basis of pharmacological requests in health care systems in Latin American countries. Peru has enacted laws and regulations that require bioequivalence studies of high health risk drugs and exemptions, based on international legislation, to be conducted in research centers accredited by the authority of Health. There is a list of 19 drugs that must demonstrate their therapeutic equivalence through in vivo or in vitro studies, of which 13 have shown bioequivalence in vivo, and 8 of those have shown bioequivalence in vitro. There is a challenge for health authorities to enforce the current legislation and an even greater challenge for pharmaceutical laboratories to demonstrate bioequivalence of multi-source drugs with the reference drug.

KEYWORDS: Bioequivalence, multi-source drug, reference drug, drug regulation, in vitro testing

INTRODUCTION

In 1984, the North American Congress approved the law of "patent protection and data exclusivity" for the pharmaceutical industry of generic drugs. This law was to initiate relative bioavailability studies to demonstrate therapeutic equivalence that would guarantee similarity in safety and efficacy of a generic (multi-source) drug with the innovative drug. This law also intended to provide accessibility to drugs that otherwise may be too expensive, therefore assuring an economic benefit (*1*, *2*). Subsequently, the World Trade Organization (WTO) and the World Health Organization (WHO) recommended that the countries that are part of these organi zations grant 20 years of exclusive sale to the innovative drug, which was extended to trade agreements with the countries where the innovative drugs originated (*1*). After the government grants the patent for the innovative drug, then the government grants licenses to similar and generic drug manufacturers so they can produce and develop other formulations in compliance with Good Manufacturing Practices (GMP) and Good Laboratory Practices (GLP) (*3*, *4*).

Innovator drugs are expensive, so they are not accessible to a large sector of the population in Peru. The use of generic drugs is usually cheaper compared to the innovator (*5*, *6*). In Peru, bioequivalence studies have been mandated by the Law of Pharmaceutical Products, Medical Devices and Health Products, Supreme Decree, which regulates the interchangeability of drugs, and by the Ministerial Resolution on the list of generic essential drugs (*7–10*). To date, the implementation process of these studies has been very slow. In this review, we will highlight studies of bioequivalent generic drugs in Peru (*5*, *11*).

*Corresponding author

METHODS

A review of the published literature on bioequivalence studies was conducted and compiled through the PubMed/Medline database. The search terms used were "bioequivalence", "therapeutic equivalence," and "in vivo and in vitro bioequivalence in Peru." The selection criteria included articles published in English and Spanish. No filters referring to the year of publication were used, and November 15, 2021 was the cut-off date.

At the same time, the web portals of the General Directorate of Medicines, Supplies and Drugs (DIGEMID) and the Ministry of Public Health of Peru were searched for regulations that require application and implementation of bioequivalence studies in Peru. Based on the collected literature, this review article has been divided into concepts, regulatory aspects, current state of studies, and future perspectives.

CONCEPTS THAT SUPPORT BIOEQUIVALENCE STUDIES

Throughout the world, three types of drugs products exist - the innovator (brand name drug), the similar (medicines manufactured by different laboratories under the same commercial name), and the generic, all of which are prepared by the pharmaceutical industries according to quality standards and regulated and authorized for prescription by the health authorities of each country (*2, 4*).

An innovator drug is the pharmaceutical product that has been developed through scientific research, going through all phases (i.e., discovery, preclinical, and phase I, II, and III clinical trials) to demonstrate its quality, safety, and therapeutic efficacy. The product is registered for the first time for commercialization with a regulatory agency (*2, 12, 13*). For an innovative product, development and commercialization requires a large financial investment, which is typically undertaken by a multinational pharmaceutical industry for 7–15 years. Because of this effort, a patent is justified and obtained for the active drug and the manufacturing process. A patent usually lasts for 20 years (*3*). The "reference" or "comparator" is the innovator product with which the generic or similar product is intended to be interchangeable, as shown in a bioequivalence study. Normally the reference is the innovator product that was registered in the country of origin (i.e., where it was patented and produced), but if the product is no longer marketed in the country, a similar drug is sought from the pharmaceutical market (*12, 13*). A "similar" drug has the same commercial name, pharmaceutical form, active ingredient, and amount of drug as the innovator drug, but no bioequivalence study has been done to establish interchangeability with the innovator drug (*6, 12, 13*).

The terms "generic" or "multi-source" have been used since 1967 to describe drugs that are pharmaceutical equivalents that may or may not be therapeutic equivalents (*6, 12, 14*). These drugs may differ in the quality of the excipients (binders, disintegrants, glidants, stabilizers, flavorings, etc.) and the manufacturing process. Generic products are manufactured by different pharmaceutical laboratories with the name of the international non-proprietary designation (INN) of the drug after the patent has expired (*6, 14*). These generic drugs will comply with international quality standards (*3*). Ideally, these drugs should be therapeutically equivalent to the innovative product and interchangeable (*12, 13*).

An interchangeable drug product is a generic or similar product that has demonstrated therapeutic equivalence with the reference or innovator by an in vivo or in vitro bioequivalence study. These studies are used to compare similar and generic/multi-source products with the innovator to show that they have the same safety and efficacy profile, thereby establishing interchangeability in clinical practice (*2, 12*).

REGULATORY ASPECTS OF BIOEQUIVALENCE

The enactment of the National Drug Policy in 2004 in Peru encouraged bioavailability studies be conducted for high-risk drugs (*15*). In 2009, Law no. 29459 (articles 4, 10, and 20) mandated that drugs must have bioequivalence studies (*7*). In 2011, Supreme Decree 016-2011-SA indicated that for the registration and re-registration of category 1 and 2 drugs, therapeutic equivalence studies should be included (*16*). On the basis of these legal antecedents, in 2015 the drug interchangeability regulation was published, the purpose of which was to receive technical and regulatory suggestions (*17*). The regulation was approved by Supreme Decree No. 024-2018-SA and was enacted on March 16, 2019 (*8*). This regulation is inspired, conceptualized, and elaborated on the basis of guidelines of the WHO, United States Food and Drug Administration (FDA), European Medicines Agency (EMA), and Canada's General Directorate for Health Products and Foods (Health Canada). In this decree, health risk criteria have been taken into account; there are instructions to carry out studies gradually over time and to use the specified method to demonstrate therapeutic equivalence. In vivo bioequivalence studies are conducted in research centers that must be certified, accredited, and meet

criteria of the Regulation of Clinical Trials of the National Institute of Health (INS). These research centers also have the supervision and technical opinion of the DIGEMID. Initially, the studies are conducted in the laboratory of the National Quality Control Center (CNCC) of the INS (*8*).

For relative bioavailability studies of a multi-source drug, DIGEMID is responsible for certifying and publishing on its website a list of reference or comparator drugs, including those with a health requirement and those from voluntary applications (*18*, *19*). The preferred choice is the innovator (or reference) product manufactured and marketed in Peru. Alternative choices are, in order of priority, the innovator product from another country; the reference drug described in the WHO list; the innovator product from a country that is a member of the International Council for Harmonization (ICH); or lastly, the leading drug in the pharmaceutical market (*8*, *20*).

Article 14 provides a long list of certain drugs that require in vivo therapeutic equivalence studies in Peru (*8*). These products include immediate-release drugs administered orally with a systemic effect, drugs with a narrow therapeutic margin and critical use, drugs where there is scientific evidence of bioavailability or bioinequivalence problems related to the active pharmaceutical ingredient (API) or its formulations (not related to dissolution problems). In vivo studies are needed when there is scientific evidence that polymorphism of the API, the excipients, and/or the pharmaceutical processes used in manufacturing influence bioavailability. In vivo studies are also required to establish equivalence through comparative clinical, pharmacodynamic, dermatopharmacokinetic studies, and/or in vitro studies for drugs designed for systemic absorption (non-oral and non-parenteral); these include transdermal patches, suppositories, testosterone gel, contraceptives inserted into the skin, and others; modified-release drugs that act by systemic absorption; fixed-dose combination drugs with systemic action where at least one of the APIs require studies in vivo; products other than solutions for non-systemic use (oral, nasal, ocular, dermal, rectal, vaginal application) designed to act without systemic absorption. Article 27 mentions drugs that do not require bioequivalence studies (parenteral [intravenous, subcutaneous or intramuscular] as an aqueous solution, elixirs, syrups, tinctures, powders for reconstitution as a solution, aqueous solutions for inhalation through nebulizers and nasal drops, aqueous solutions for optic or ophthalmic use, and pharmaceutical forms in gases), but these drugs must meet specifications of the corresponding pharmacopoeia or manufacturing laboratory's own technique when appropriate (*8*). DS No 024-2018-SA-MINSA requires in vitro bioequivalence studies of lamivudine (150 and 300 mg tablets), zidovudine (100 capsules and 300 mg tablets), lamivudine and zidovudine combination therapy (150 and 300 mg tablets), and diazepam (10 mg tablets) (*8*). Subsequently, Ministerial Resolution No. 404-2021 (March 19, 2021) expanded the list of drugs for which therapeutic equivalence must be demonstrated through in vivo or in vitro studies (Table 1) (*8*, *19*, *21*, *22*).

Regarding the legislation on relative bioavailability studies in Latin American countries (Argentina ANMAT3185/99; Brazil ANVISA 987/99; Colombia INVIMA 1400/2001; Costa Rica SINALVI N° MS-CTI-001-2021; Chile MINSAL 500/12; Ecuador R. ARCSA -DE- 015-2018JCGO; Mexico NOM-177-SSA1-2013; Paraguay R.N° 077/18; Uruguay Decree N° 12/007; Venezuela R.N° 212-2006), all include drugs with a narrow therapeutic margin (they have very close therapeutic and toxic concentrations). Also included are drugs indicated for serious conditions (antibiotics, anticonvulsants, antineoplastics, antiretrovirals, antiarrhythmics, digitalis, immunosuppressants, among others), drugs with incomplete absorption, low solubility, instability, and those with evidence of bioavailability problems.

CURRENT STATUS OF BIOEQUIVALENCE STUDIES

Relative bioavailability studies demonstrate the bioequivalence of a generic drug (multi-source) in comparison with the reference, and therapeutic interchangeability is established in clinical practice. However, for a certain group of drugs, therapeutic equivalence is established through in vitro bioequivalence studies, based on the criteria of the Biopharmaceutical Classification System (BCS). Solid oral immediate-release medications must meet one of the following BCS criteria (*8*, *10*, *22–24*).

- Class I (high solubility and high membrane permeability): very fast or rapid dissolution with release of more than 85.0% of drug in 15 or 30 min, respectively. Excipients criterion: the drugs should not contain excipients that affect the absorption of the drug.
- Class III (high solubility and low membrane permeability): very fast dissolution with release of more than 85.0% of drug in 15 min. Excipients criterion: the test drugs must contain the same excipients in similar amounts as the reference product.

Table 1. Medicines that Require In Vivo and/or In Vitro Bioequivalence Studies in Peru (8, 19, 21, 22)

Category	API	Dosage Form	Dose	Type of study
Anticonvulsants	Valproic acid, semisodium valproate, and sodium divalproate	Extended-release tablet	250 and 500 mg	In vivo
	Valproic acid, semisodium valproate, sodium divalproate, and sodium valproate	Delayed release tablet, coated gastro-resistant tablet, and enteric-coated tablet	250 and 500 mg	In vivo
	Carbamazepine	Tablet	200 mg	In vivo
	Sodium phenytoin	Capsule	100 mg	In vivo
	Lamotrigine	Compressed tablet	50 and 100 mg	In vivo
	Lamotrigine	Dispersible or chewable tablet	50, 100, and 200 mg	In vivo
	Levetiracetam	Extended-release tablet	500 mg	In vivo
	Oxcarbazepine	Tablet	300 and 600 mg	In vivo
Antiarrhythmics and Digitalis	Verapamil hydrochloride	Coated tablet	80 mg	In vivo
	Digoxin	Tablet	0.25 mg	In vivo
Anticoagulants	Warfarin sodium	Tablet	5 mg	In vivo
Bronchodilator	Theophylline	Sustained-release tablet	250 mg	In vivo
Hormones (thyroid)	Levothyroxine sodium	Tablet	25, 50, 75, 100, 125, and 150 mcg	In vivo
Immunosuppressants	Azathioprine	Coated tablet	50 mg	In vivo
	Mycophenolate mofetil	Capsule and coated tablet	250, 250, and 500 mg	In vivo
	Tacrolimus	Capsule	0.5, 1, and 5 mg	In vivo
Psychopharmaceutical	Lithium carbonate	Tablet	300 mg	In vivo
Other	Topiramate	Coated tablet	25, 50, and 100 mg	in vitro
	Levetiracetam	Coated tablet	500 and 1000 mg	in vitro
	Levodopa + carbidopa	Tablet	25 and 250 mg	in vitro

API: active pharmaceutical ingredient.

Table 2 lists the relative bioavailability studies that demonstrate bioequivalence, conducted by pharmaceutical laboratories in accordance with regulations and laws. Table 3 lists in vitro bioequivalence studies conducted by researchers from various Peruvian universities (*5*, *10*, *23–28*).

FUTURE PERSPECTIVES

Despite the efforts and dedication of regulators to implement bioequivalence studies in Peru, progress has been slow. Health authorities and university researchers encourage and promote the performance of bioequivalence studies, and laboratory pharmacists comply with regulations for registration and re-registration of their pharmaceutical products. All this effort makes it possible to have a greater number of bioequivalent generic drugs that fulfill their social good, that is, to be accessible and available to the population with fewer economic resources (*3*, *14*). By having bioequivalent multi-source drugs, Peruvian medical specialists can prescribe them in clinical practice to demonstrate interchangeability with the innovative drug for a specific disease (*15*). Bioequivalence and pharmacogenomic studies are essential in the Peruvian population, who have tricontinental (European, African, Asian) and Latin American ancestry (*CYP2D6*, *CYP2C9*, *CYP3A4* genes, and others). Bioavailability may vary according to genetics, leading to personalized doses to optimize pharmacological therapy (*29*, *30*).

CONCLUSIONS

Legislation has been enacted to mandate in vitro and in vivo bioequivalence studies in Peru; however, there is still a challenge for health authorities to enforce current legislation and an even greater challenge for pharmaceutical laboratories to demonstrate bioequivalence of multi-source drugs with reference drugs. Having bioequivalent (quality, efficacy, and safety) medications (multi-source and similar commercial brand) guarantees interchangeability in clinical practice with the reference medication (efficiency).

FUNDING

This work was supported by the Molecular Pharmacology Society of Peru and the International Research Network in Pharmacology and Precision Medicine, Human Medicine School, of the San Ignacio de Loyola University, Lima, Peru.

Table 2. In Vivo Bioequivalent Drugs Approved by DIGEMID in Peru (8, 17)

In Vivo Bioequivalent	Dosage Forms (Dose)	Brand Name, Manufacturer (Certificate Number)	Indication
Cyclosporine	Soft capsule (100 mg)	Supramunn, Teva Peru SA (EE03968)	Immunosuppressive drug
Carbidopa+ Levodopa	Tablet (25 and 250 mg)	Trade Name, Sanofi Aventis Del Peru SA (EE1466)	Parkinson's disease
Cilostazol	Tablet (25 and 250 mg)	Cilosvitae, Galenicum Health Peru SAC (EE01870)	Phosphodiesterase 3 inhibitor, vasodilator, and antiplatelet agent
Dutasteride + Tamsulosin	Prolonged-release capsule, hard (0.5 and 0.4 mg)	Bripost-TD, QM Pharma Quality Medicine SAC (EE09137).	Benign prostatic hyperplasia
Escitalopram	Coated tablet (20 mg)	Etalpram, Eurofarma Peru SAC (EE04274)	Antidepressant (selective serotonin reuptake inhibitor)
Etoricoxib	Coated tablet (90 and 120 mg)	Movicoxib, Deutsche Pharma SAC (EE09486 and EE09380)	Non-steroidal anti-inflammatory drug (selective COX-2 inhibitor)
Ibrutinib	Hard capsule (140 mg)	Binap, Varifarma SA (EE07537)	Bruton's tyrosine kinase inhibitor (BTK), antineoplastic
Lamivudine + Zidovudine	Coated tablet (150 and 300 mg)	Trade Name, Seven Pharma SAC (EE02372)	Trade Name, Seven Pharma SAC (EE02372)
Olanzapine	Coated tablet (10 mg)	Olazanvitae, Galenicum Health Peru SAC (EE01928)	Atypical antipsychotic drug
Quetiapine	Coated tablet (25 mg)	Trade Name, Sanofi Aventis Del Peru SA (EE00899)	Atypical antipsychotic drug
Rosuvastatin	Coated tablet (20 mg)	Xuniro, Sanofi Aventis Del Peru SA (EE00507 and EE00027)	Statin inhibitor of HMG-CoA reductase
Sitagliptin	Coated tablet (100 mg)	Sitavitae, Galenicum Health Peru SAC (EE09486)	Antihyperglycemic drug that belongs to the class of dipeptidyl-peptidase 4 inhibitors (DPP-4 inhibitor)
Ceftriaxone	Iny (1 g)	Betasporina, Medifarma*; Cefalogen, Eurofarma Peru SAC* (EE04413 and EE05383)	Antibiotic

DIGEMID: General Directorate of Medicines, Supplies and Drugs.
**These laboratories have voluntarily accepted bioequivalence studies, since powders for solution for injection do not require bioequivalence studies*

Table 3. In Vitro Bioequivalent Generic Drug Comparison Studies from Peru

API	Results and Conclusions	Reference
Amlodipine	Amlodipine generics are in vitro equivalent to the reference drug.	*24*
Amoxicillin, Doxycycline, Fluconazole	The generics doxycycline and amoxicillin are equivalent. Fluconazole was not equivalent.	*2*
Carbamazepine	Generics of carbamazepine are equivalent.	*10*
Diazepam	Generic diazepam not equivalent.	*26*
Phenytoin	Phenytoin generics are not equivalent.	*23*
Glibenclamide	Generics of glibenclamide are equivalent.	*5*
Ibuprofen	Generic of ibuprofen are equivalent.	*27*
Paracetamol, Chlorphenamine, Phenylephrine	The three types of generics are not equivalent.	*28*

API: active pharmaceutical ingredient.

CONFLICTS OF INTEREST

The authors disclosed no conflicts of interest related to this article.

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dx.doi.org/10.14227/DT290422P228

Book Review: "Analytical Testing for the Pharmaceutical GMP Laboratory"

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Analytical Testing for the Pharmaceutical GMP Laboratory (Hunyh-Ba, K.; Holberg, W.; Lin, J.; Ng, L. L.; Gray, V. A.; Famili, P.; Cleary, S.; Wiley, 2022. ISBN 9781119120919) is an excellent and comprehensive book. It includes extensive, concrete instructions and examples of key documents. This book is a valuable resource for individuals entering the pharmaceutical industry, especially for those following an analytical, chemistry and controls (CMC), or development path. It is particularly useful for recent graduates and professionals working in small companies where access to experienced colleagues may be limited.

The book begins with a review of pharmaceutical laboratory regulations, with an emphasis on U.S. Food and Drug Administration (FDA) and International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use requirements and their roles in drug development, registration, and control. Kim Huynh-Ba continues with a discussion on Good Manufacturing Practices (GMPs) and the roles and responsibilities of the quality control unit. Areas addressed include personnel qualification, instrument qualification, testing programs for release and stability, and documentation. The book also provides an overview of pharmaceutical quality systems.

Chapter 3 introduces several analytical techniques used in the GMP laboratory, including both chemical and microbiological testing. Successful use of analytical techniques requires good statistical control, which is the subject of chapter 4. Chapters 5 and 6 provide important guidance and practical advice for development, validation, and transfer of these analytical techniques.

Dissolution testing is unique to the pharmaceutical industry, so this technique warrants an entire chapter. This chapter covers not only the basics of United States Pharmacopeia (USP) apparatuses and method development, but also provides extensive practical information to help the reader avoid many of the potential pitfalls encountered in dissolution testing.

The book concludes with a chapter on the analytical laboratory, including critical subjects related to the pharmaceutical data such as documentation systems, stability programs, and LIMS/electronic data, and quality control.

Overall, *Analytical Testing for the Pharmaceutical GMP Laboratory* is a valuable training tool and reference. It is highly recommended as a complete and comprehensive introduction to testing in the GMP laboratory.

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dx.doi.org/10.14227/DT290422P230

Dissolution Best Practices and International Harmonization - AAPS Workshop Report

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The AAPS In-Vitro Release and Dissolution Testing (IVRDT) Community and Stability Community jointly organized the virtual workshop "Dissolution Best Practice and International Harmonization," held on August 16th, 2022. The workshop was designed to bring awareness to differences in dissolution testing and acceptance criteria between international pharmacopoeias, discuss how to address these differences, develop science-based dissolution design strategies, and meet the needs of the international market. The workshop consisted of two sessions – dissolution in pharmacopeias and dissolution best practices.

Many compendial procedures and chapters have been established, including *United States Pharmacopeia* (USP), *European Pharmacopoeia* (EP), and *Japanese Pharmacopoeia* (JP), to establish standards for quality control of drug products, e.g., dissolution testing. Although the International Conference on Harmonization (ICH) has expended a great effort to standardize technical measurements of pharmaceuticals for human use, differences exist among the pharmacopeias, especially from new ICH members, such as Chinese Pharmacopoeia (ChP). There are differences in acceptance criteria and specifications that impact the dissolution design strategy and drug release profile. Considerable retooling of the dissolution methods and specifications may be required when a company plans to release product in other countries that have different standards for dissolution testing.

PART 1: DISSOLUTION IN PHARMACOPEIAS

The first session was moderated by Xujin Lu, PhD (Bristol-Myers Squibb, New Brunswick, NJ, USA). The first speaker was Mark Alasandro, PhD (MZA Pharmaceutical Consulting, San Diego, CA). The talk title was "Dissolution Best Practices – Understanding the acceptance criteria in different Pharmacopeia." He explained the differences in dissolution testing requirements provided in the ChP, JP, EP, and USP pharmacopeias (*1*). He also shared the results from AAPS survey on awareness of these differences along with strategies to address these differences. These strategies are critical to avoid last minute retooling of methods, specifications, and delaying product launch. He explained the need for a globally accepted dissolution method with a single specification. Such a method would streamline generation of dissolution data to support formulation, process, and raw material changes globally. Coupling such a method with the knowledge active pharmaceutical ingredients (API) solubility, permeability, and pharmacokinetics would also help secure biowaivers. This knowledge can also be used to build Bayesian and other statistical modeling approaches to predict the impact of changes on product performance, safety, and efficacy.

For the dissolution test, there are similarities between ChP, USP and ICH Q4b, such as dimensions of 1-liter dissolution vessel and paddle size, but there are many differences.

- The definition of the ChP Q differs from the USP Q.
  - The Q_{ChP} is the same as USP Q + 5%, so the dissolution specification for China is listed as Q_{ChP} + 5%, whereas, for the US and other ICH region it is listed as Q.

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- ChP only has 2 stages of testing whereas USP and ICH have 3 stages.
  - ChP maintains the original two-stage approach of the JP and EP even though JP and EP have aligned with the USP three-stage approach as noted in ICH Q4B. USP has always had a 3-stage approach since the first publication of the USP General Chapter Dissolution <711>.
- The ChP stages of testing are *Stage 1*: mean of 6 units must be ≥ Q_{ChP} and no two units can be less than Q_{ChP} – 10%; if one unit is < Q – 10%, then go to stage 2 and test another 6 units. *Stage 2*: mean of 12 units is ≥ Q_{ChP} and not more than three units are < Q_{ChP} – 10% and only one unit is < Q_{ChP} – 10% but ≥ Q_{ChP} – 20%.
- Total number of units tested is 12, not 24 as allowed in the USP, JP and EP. Same applies for extended and delayed released formulations, where only 12 total units can be tested.
- For performance verification testing, ChP uses salicylic acid not prednisone tablets.
- The use of enzymes is not allowed by the ChP to address gelatin capsule shell crosslinking.
- ChP has only adopted the 1-liter vessel, not 2- or 4-liter vessels (which are not part of ICH).

Based on the AAPS survey, 50% of the AAPS community surveyed were unaware of the differences and about 30% had problems filing in China. Some of these problems were addressed by adopting the ChP guidance, or by working with the regulatory agency to gain acceptance of their USP-based method or use a modified USP approach, or by showing that their company's data meets ChP guidance using statistical analysis.

Overall, ChP requirements are more stringent if the data shows variability and ICH Q4B stage 2 and 3 testing is needed. Some regulatory flexibility may be allowed through discussions about the data and specifications with regulators. Moving forward, there may be more alignment with the ICH Q4B in the next 2025 ChP edition. Other opportunities for further discussion include the use of in vitro in vivo correlation, physiologically based biopharmaceutical modeling (PBBM), biowaivers, enzymes, and more.

The second speaker was Kevin Moore, PhD (USP, Rockville, MD, USA) who spoke on the topic, "Pharmacopeial Convergence and Harmonization." He is the USP delegate to the ICH Assembly, and in his talk introduced the role of USP in international harmonization with a specific focus on the Pharmacopeial Discussion Group (PDG) and ICH. The talk focused on describing USP's approach to pharmacopeial convergence and harmonization as critical tools to promote the alignment of quality standards to ensure consistent global access to quality medicines for the benefit of public health. An overview was provided for the PDG, which brings together USP, EP, and JP, with WHO as an observer in the harmonization of broad impact general chapters and excipients monographs, with a total of 31 general chapters and 60 excipient monographs on the PDG workplan. In addition, the history of interaction between PDG with ICH was provided, with a synopsis of the activities of the ICH Q4B Expert Working Group, which evaluated regulatory interchangeability of 15 general chapters on the PDG workplan. Also, the talk chronicled the first major reforms of the PDG (in its 32 years of existence) to integrate additional pharmacopoeias from regions not yet represented through the establishment of a pilot for expansion, set to begin this fall. This milestone decision marks a critical step in the PDG's commitment to expanding recognition of harmonized pharmacopeial standards. Lastly, the history of dissolution harmonization in PDG and interchangeability of the chapter through ICH Q4B was presented in detail, including the example of how harmonized text is distinguished from local requirements in the USP text and how PDG Sign Off cover sheets are written, which are publicly available and provide information on non-harmonized and local requirements in PDG pharmacopeias. Detailed information on the PDG including workplan, purpose, process, statement on harmonization policy, and the PDG working procedure and interaction with ICH Q4B can be found on the USP website at https://www.usp.org/harmonized-standards/pdg.

The third speaker was Margareth Marques, PhD (USP, Rockville, MD, USA) who spoke on "USP General Chapter Dissolution <711>." This chapter describes the apparatus and test conditions for dissolution of the most common pharmaceutical dosage forms (tablets, capsules, and suspensions). This chapter is partially harmonized with the EP and JP.

The USP national text is easily identified by the symbol: ♦,♦; text within these symbols is applicable to USP only. One example of USP national, not harmonized, text is the section "For Dosage Forms Containing or Coated with Gelatin," where use of enzymes in the dissolution medium when there is evidence of crosslinking in

gelatin is described. Another example is the use of USP Prednisone Tablets for the qualification of the dissolution apparatus 1 (basket) and apparatus 2 (paddle), which is applicable only to USP. Also, the text describing USP apparatus 3 (reciprocating cylinder) has a footnote stating that this apparatus is not accepted by the JP. The USP has hundreds of individual monographs for pharmaceutical dosage forms. These monographs have dissolution test conditions that are specific for products approved for the USA market, with few exceptions.

The dissolution, disintegration, or drug release test conditions, including the acceptance criteria, in any USP monograph are the conditions approved by FDA for products marketed in the USA. There are a few exceptions in which the monographs were developed upon request from the WHO. One example is the monograph for zinc sulfate tablets. This product is not approved for the USA market, and the monograph was developed based on a product approved for the European market.

USP <711> contains the acceptance criteria used in the evaluation of dissolution results for different release mechanisms (immediate, delayed, and extended release). If a particular product was approved with a product-specific acceptance table, this table is included in the particular monograph. Some examples can be found in the monographs for Clarithromycin extended-release tablets, Divalproex sodium extended-release tablets, and Extended phenytoin capsules. Two useful tools that can be a starting point in the development of dissolution tests are the FDA Dissolution Methods database, available at https://www.accessdata.fda.gov/scripts/cder/dissolution/index/cfm, and the USP Dissolution Methods database, available at https://www.usp.org/resources/dissolution-methods-database.

Proposals for revisions to any USP general chapter or monograph are published in *Pharmacopeial Forum*, available free of charge at www.uspnf.com. New proposals are posted bimonthly and are open for public comment for 90 days. Each proposal, including those being harmonized with EP and JP, has a brief explanation of the reasons for revision. Comments and suggestions for revisions to any part of the USP–NF are welcome. Comments should be supported by data, have a scientific justification, and be an improvement to the standard.

The final speaker of this session was Fasheng Li, PhD, (Pfizer, Groton, CT, USA). His topic was "USP <711> vs ChP (0931) dissolution acceptance criteria comparison and challenges to the industry." The in vitro dissolution test has been routinely used by pharmaceutical companies for commercial productions of solid dosage forms for quality control and prediction of in vivo drug release. However, there are large gaps between the different pharmacopeias with respect to dissolution test methods and acceptance criteria.

The presentation focused on the comparisons the dissolution test acceptance criteria between USP <711> and ChP (0931) using Monte Carlo simulation modelling. Operating characteristic curves were used to evaluate the probabilities of satisfying the respective acceptance criteria in two seemingly distinct compendial guidances.

When comparing USP <711> and ChP (0931) for testing the same product, ChP (0931) criteria are more stringent than USP <711> if using the same Q value for the same product. It was suggested that a standard deviation threshold for a drug product batch might be established. For results below that threshold, it would not be necessary to test against other compendial criteria.

The session ended with a panel discussion joined by Dr. Baoming Ning from the Chinese National Institute for Food and Drug Control. There was a discussion on why ChP only has two-stage testing. Dr. Ning explained that ChP adopted the EP and JP at a time when only two-stage testing was allowed. Although EP and JP have since adopted a three-stage approach, ChP has not. Dr. Ning also shared that ChP is making efforts to align with ICH. The agency has transformed and implemented a number of ICH guidelines by recommending them and publishing the Chinese version of original ICH guidelines and by assigning experts to participate in the in-depth coordination of ICH issues, including dissolution issues. ChP is open for discussion and are flexible to alternative approaches.

PART 2: DISSOLUTION BEST PRACTICES

The second part of the workshop was moderated by Yan Wu, PhD (Merck & Co., Inc, Rahway, NJ, USA). The first speaker was Andreas Abend, PhD (Merck & Co., Inc, Rahway, NJ, USA), who spoke on the topic of "Designing a Science Based Approach." Pharmaceutical scientists perform dissolution testing primarily to 1) rank formulation prototypes with varying compositions and/or made under different processing conditions, 2) assess product sameness as part of quality control; or 3) gauge the impact of formulation and manufacturing changes on product quality (*2*). These tasks often require testing under a variety of experimental conditions. The selection of the appropriate methodology is usually based on drug substance physicochemical properties, formulation composition, manufacturing/process conditions, and

drug product design (i.e., immediate, delayed, or extended release, etc.). There are no regulatory provisions restricting the choice or experimental conditions when dissolution is used in support of formulation candidate selection (*3*). Hence, companies can choose an experimental method based on prior knowledge or publications found in peer literature that are deemed appropriate to drive rational formulation and process selection. Approaches currently used in industry range from simple multimedia dissolution experiments performed in standard compendial dissolution apparatus to highly complex transfer models like the Gastro-Intestinal Simulator (GIS) or TNO Gastro-Intestinal Model (TIM). At the beginning of product development, where formulation prototype performance is solely evaluated in vitro or in preclinical species, the risk of making poor formulation or process choices as a result of relying on tests with unknown in vivo relevance is entirely with the development teams.

Once a formulation is used in the clinic or when the product is on the market, consistent product performance is critical. As a result, companies pivot their dissolution strategy towards methods and experimental conditions that ensure product quality as well as acceptance of a single specification in a complex and misaligned global regulatory environment. During market application review, many regulatory agencies challenge dissolution specifications for products containing poorly soluble drug substances if a company fails to demonstrate the ability of the specification to reject product that may not perform in patients as claimed in the product label. In an effort not to delay product approval and launch, companies often file specifications that are sensitive to small variations in materials attributes that are unlikely to impact in vivo performance. This practice often results in unnecessarily tight manufacturing process controls. Furthermore, companies may have to accept different dissolution specifications proposed by different agencies, and as a result they may have to apply different acceptance criteria for the same product (*4*). This dissolution specification development and filing approach, which until recently was common practice in the industry, is not considered "best science," as the proposed method and acceptance criterion are not capable of reliably distinguishing good from bad product, which is exactly what a specification is intended to do. Depending on drug substance physicochemical properties and formulation complexity, industry is encouraged to develop clinically relevant dissolution specifications (CRDS). A clinically relevant dissolution specification requires the dissolution method to demonstrate that changes in rate and extent of in vitro dug release produces similar changes in rate and extent of in vivo (PK) release of the drug into the systemic circulation. Thus, the specification is based on acceptable in vivo performance as opposed to some manufacturing parameter that is assumed to be in vivo relevant.

Assessing the impact of manufacturing (i.e., formulation and or process) changes on product quality for approved drugs is highly regulated, and the battery of tests to justify these changes depend on the nature of the change and drug substance solubility and permeability, i.e., the Biopharmaceutics Classification System (BCS). The level of dissolution testing a company needs to perform depends on the expected impact on product quality. Accordingly, for minor changes (unlikely to have in vivo impact) falling within the scope of current guidance, passing the approved dissolution specification may be sufficient. For moderate changes (e.g., there may be an impact in vivo performance), dissolution testing often requires dissolution profile similarity assessment. The latter may range from assessing profile similarity using the approved dissolution method or testing in as many as four pH levels of aqueous media and water (without surfactant). However, as with non-clinically relevant dissolution specifications, there's no guarantee that such dissolution profile assessments are indicative of acceptable or unacceptable in vivo performance. Here again, a clinically relevant dissolution method provides the link between in vitro rate and extent of drug release and in vivo performance and should therefore replace the above-mentioned multimedia assessment.

The next speaker in the second session of the symposium was Tessa M. Carducci, PhD (Merck & Co., Inc., Rahway, NJ, USA), who gave a talk on "Global Best Practices." As more pharmaceutical companies are filing drug products globally, Dr. Carducci emphasized that there is a strong business driver for universal acceptance of quality control dissolution methods and specifications for products in global markets. Additional sampling, results assessment, and/or testing an additional method extends the product release time and analysts needed, also adding supply risk and complexity. Optimizing the chance of global acceptance of the dissolution method often involves selecting the most discriminating method without sacrificing method robustness. Although adhering to multiple country-specific guidelines can be overwhelming, there are common themes underlying the principles governing dissolution method development in various markets, such as appropriate discriminating power (*5*–*7*). Furthermore, more markets are embracing scientific justifications including clinically relevant arguments (*7*). Pharmaceutical companies can help continue to drive

global acceptance by presenting innovative, science-based, and clinically relevant justifications to the agencies.

Dr. Carducci presented a case example for a Biopharmaceuticals Classification System (BCS) class IV immediate-release tablet made by direct compression. Although the equilibrium solubility of the active compound is low in pH 6.8 media, the dissolution is rapid due to high apparent solubility of the API, and the supersaturated solution is stable. Two main quality control dissolution method options were considered, either 0.1 N HCl or pH 6.8 buffer. Both options demonstrate robustness for routine commercial testing, and both are sensitive to process parameters and considered discriminating; the pH 6.8 method has greater sensitivity to process parameters and is additionally sensitive to the API form. Although this method could be considered over-discriminating due to there being no risk of API form change in the drug product even on stability, the 0.1 N HCl method could be seen as under-discriminating. Therefore, the pH 6.8 method was proposed universally to ensure global acceptance as it is more discriminating without having execution risks in supply. As with the dissolution method proposal, Dr. Carducci explained that we can optimize our chance of global acceptance of the dissolution specification by selecting the most discriminating specification per relevant regulatory guidances or preferences and providing a strong justification that includes linkage to clinical relevance. Another case example was given where the specification following the EMA reflection paper would be set at 30 minutes based on the dissolution of batches used in pivotal clinical studies, but a tighter specification at 20 minutes was proposed to align with the FDA expectation for the specification to be set where 80% release is achieved (*3*, *5*). The specification at 20 minutes does not significantly increase risk of failing acceptable batches as compared to 30 minutes and has increased chance of global acceptance.

Dr. Carducci closed with a proposal for leveraging the procedures in ICH M9 as a path towards a universal multimedia dissolution procedure for demonstrating in vitro dissolution comparison for both post-approval product changes and changes made during development (*8*).

The next speaker was Beverly Nickerson, PhD (Pfizer, Groton, CT, USA), and the topic was "Dissolution Testing with Apex Vessels." Dr. Nickerson highlighted challenges associated with coning during dissolution testing and the benefits of using apex vessels to address these issues. Coning is an artifact that may be observed during dissolution testing of some solid oral dosage forms due to insoluble excipients in the formulation. This can lead to the presence of a cone of dense undissolved excipients at the bottom of the dissolution vessel under the paddle. The cone of material prevents dissolution of drug that is trapped in the cone.

PEAK vessels (now commonly referred to as apex vessels) were introduced by VanKel in the 1990s to minimize the effect of coning (*9*). These vessels have an inverted cone at the bottom of the vessels to prevent material from accumulating under the paddle. Despite the availability of these vessels for so many years, there are very few methods listed in the FDA dissolution database that use PEAK or apex vessels, and there is continued reluctance by companies to use the vessel due to fears of lack of regulatory acceptance.

Dr. Nickerson also discussed a *Stimuli* article that was published in *Pharmacopeial Forum* in collaboration with members of IQ Dissolution Working Group, AAPS In Vitro Release and Dissolution Testing Community, and apex vessel manufacturers (*10*). The goal of the article was to seek acceptance of the apex vessel as an alternative to the standard 1-L vessel to be used when scientifically justified. This article compared apex vessels from various major manufacturers through an interlaboratory study and through computational fluid dynamics modeling. In addition, specifications and qualification procedures for apex vessels were proposed. Dr. Nickerson presented an example of a project she worked on that included the use of apex vessels to develop a discriminating and robust method for an immediate-release tablet.

The next speaker was Bryan Crist (DissoAssist, Wilmington, NC, USA), and his topic was "Dissolution Apparatus Qualification Criteria." Mr. Crist provided elements of dissolution apparatus performance qualification as defined by the US FDA, USP, ASTM, and various international pharmacopeias. Reflecting on best practices for analytical instrument qualification (AIQ) from USP general chapter <1058> Analytical Instrument Qualification, he differentiated between the holistic qualification requirements of the USP Performance Verification Test (PVT) included in USP <711> and modular qualification requirements of the enhanced mechanical qualification (eMQ) procedure in ASTM-E2503-13 for the basket and paddle dissolution apparatus. Elements of dissolution apparatus qualification parameters contained in the ChP (0931) were also compared to USP and ASTM specifications and tolerance.

A historical perspective was provided for the various apparatus qualification procedures along with review of

advantages and limitations of the USP PVT and ASTM eMQ. Mr. Crist ended with reminders that either the PVT or eMQ will satisfy cGMP requirements for performance qualification of the dissolution apparatus but cautioned that the goal of a proper AIQ was that the apparatus remain in a qualified state between performance qualification intervals. There were three advantages that the eMQ approach had for accomplishing this by 1) reducing the intervals between periodic qualification based on risk; 2) requiring analyst's documentation of observational checks prior to each run; and 3) replacing damaged or out-of-specification components with certified components.

The final speaker for this session was Piero Armenante, PhD (New Jersey Institute of Technology, Newark, NJ, USA). His topic was "The Hydrodynamics of the USP Apparatus 1 (Basket Apparatus)." He presented results of experimental work that he and his students conducted to study in detail the hydrodynamics of the USP apparatus 1. They used particle image velocimetry to determine the fluid velocities in the dissolution vessel on a vertical central plane through the basket and on a number of horizontal planes for three different basket rotational speeds and with different mesh openings (*11*, *12*). They found that flow field was dominated by the tangential velocity component and was approximately symmetrical in all cases. However, despite all precautions taken, small flow asymmetries were observed in the axial and radial directions which appears to be an unavoidable characteristic of the fluid flow in apparatus 1. The magnitudes of axial and radial velocity components varied significantly with location in the vessel, basket rotational speed, and mesh opening, but were always much lower to the tangential velocities. Interestingly, a small vertically angled jet emanating radially near the top edge of the basket was observed. This jet contributes significantly to the vertical recirculation of the fluid inside the vessel and especially to the flow through the basket and around the dosage form within, having major implications on the drug dissolution rate. The results of this work provide insight into the flow field inside USP apparatus 1 and how operating and geometric variables affect the system hydrodynamics and hence the dissolution process.

The session ended with a panel discussion where there was some discussion on strategies to support formation changes during development, such as going from drug in a capsule to a capsule formulation and then to a tablet formulation for phase 3. Depending on the specific case, possible strategies include comparison of the dissolution profiles to support the new formulation, an IVIVC study, or small (e.g., 12 patients) in vivo comparability study.

SUMMARY

The recordings of the meeting, including panel discussions, are available on the AAPS website. The workshop was well received, with more than 60 people in attendance and active participation in two panel discussion sessions. The workshop accomplished its goal as a forum to learn and discuss strategies for dealing with different dissolution methods and acceptance criteria in different pharmacopeia, developing the dissolution method and setting specifications with global acceptance in mind. These strategies will benefit the industry for global marketing effort and enhance international best practices by presenting innovative, science-based, and clinically relevant dissolution justifications to the agencies.

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Q+A Question & Answer Section

*The following questions have been submitted by readers of Dissolution Technologies. Margareth R. Marques, Ph.D., and Mark Liddell, Ph.D., United States Pharmacopeia (USP), authored responses to each of the questions. *Note: These are opinions and interpretations of the authors and are not necessarily the official viewpoints of the USP. E-mail for correspondence: mrm@usp.org.*

Q Is it possible to obtain dissolution results that are higher than the assay results for a particular product batch?

A Yes, it is. First, the filter and cleaning methods used should be properly evaluated to ensure that there is no placebo interference or carryover issues and that the sampling and sample filtration were carried out in the proper way. If all these parameters were found to be appropriate, one possible reason for dissolution results being higher than the assay results is that the dissolution values reflect variability better captured by uniformity of dosage units for the batch. Although the assay value for a given batch reflects the average content of several individual dosage units, as only one dosage unit is introduced in each dissolution vessel, there is a chance that a particular unit could be close to the upper limit of the range of uniformity of dosage unit values observed.

Q To define the sink condition in a dissolution test, which parameter should be evaluated: "Apparent solubility - Physical Assessment of Solubility" or "Methods for Determination of Equilibrium Solubility (saturation shake-flask method)"?

A Sink condition is defined as at least three times the volume of dissolution medium needed to obtain a saturated solution considering the highest product dose. The shake flask method, which is used to determine the equilibruim solubility in a specified solubility medium, is the easiest procedure for the determination of the equilibrium solubility. The solubility medium should be considered when defining sink conditions for the proposed dissolution meduim. Apparent solubility is typically used to characterize the pure drug substance. See USP general chapters <1236> Solubility Measurements and <1087> Intrinsic Dissolution – Dissolution Testing Procedures for Rotating Disk and Stationary Disk.

Q Can the immersion cell apparatus be used to carry out the in vitro permeation test (IVPT) of semisolid dosage forms using membranes of natural origin (e.g., pig skin)?

A The immersion cell is most commonly used with synthetic membranes due to the robustness of the synthetic membranes. The recommendation is to use the vertical diffusion cell or the horizontal flow-through cell when using biological membranes. Human skin obtained from cosmetic surgeries is preferred for this type of test. A major revision to the USP general chapter <1724> Semisolid Drug Products – Performance Tests was developed to include the IVPT procedures and equipment. See *Pharmacopeial Forum* 48(3) May – June 2022, available free of charge at www.uspnf.com. This revision discusses various proposed test conditions for in vitro permeation tests.

Q The USP general chapter <711> Dissolution contains instructions on how to run the Performance Verification Test (PVT) only for 1-L vessels. Can this procedure be used with 2-L vessels or is only mechanical verification necessary for these larger vessels?

A The PVT was developed for vessels having a nominal capacity of 1 L. The collaborative study used to set the PVT limits is conducted on instruments with 1 L vessels, so the PVT limits do not apply to dissolution systems with alternative vessel dimensions and volumes. A typical approach is to setup the instrument and perform the PVT with 1 L. Then, set up the equipment with your 2-L vessels provided that the mechanical calibration parameters (i.e., vessel alignments, paddle height, etc.) with the 2 L vessels installed meet the requirements stated in <711>, then it is assumed that the instrument will perform as expected.

Q The deaeration procedure described in the USP <711> Dissolution states that "the measured vacuum should be less than 100 mbar." Does it mean the vacuum pressure range is 0–100 mbar or the opposite?

A Yes, the pressure of the container under vacuum should be in the range from 0–100 mbar. The applied vacuum should below 100 mbar for the entire 5 minutes.

Q What is the role of sodium lauryl sulfate (SLS) in the dissolution test in the USP monograph for simvastatin? What will be the impact if there is an excess of 50 g in preparation

of the dissolution medium?

A Simvastatin is a poorly soluble drug. SLS, a surfactant, is used to increase the solubility of simvastatin. The dissolution test for any formulation containing simvastatin is likely to be formulation-dependent. The validated dissolution test should be discriminative for the critical quality attributes of the formulation. A higher amount of SLS may lose the discriminatory power of the dissolution test. Whatever the concentration of SLS used, the discriminatory power of the dissolution method will have to be verified as part of the dissolution method validation.

Q **We are developing a dissolution test for tacrolimus capsules. In the USP monograph for this product, the dissolution medium uses hydroxypropyl cellulose. Is there any information about what specific type (viscosity grade/ molecular weight) of hydroxypropyl cellulose that we can use?**

A The dissolution test for tacrolimus capsules is formulation-dependent. Each formulation is going to have its own specific and discriminative dissolution test. This is the reason for multiple dissolution tests in the USP monograph for tacrolimus capsules. Each dissolution test in this monograph is specific for a particular product approved by FDA for the US market. A dissolution test that is discriminative for the critical quality attributes of your formulation should be developed and validated. You can find the specification of any reagent mentioned in USP–NF in the Reagent Specifications section where all reagents are listed in alphabetical order. Hydroxypropyl cellulose is used to minimize the adsorption of tacrolimus into the glass walls of the dissolution vessels.

Q **As per USP <711> Dissolution, "The assembly consists of the following: a vessel, which may be covered, made of glass or other inert, transparent material." Usually, we are using glass and polycarbonate vessels for drug product dissolution analysis. Which one is recommended by USP or is a glass vessel mandatory?**

A As there are drug substances that adsorb in glass and others in plastic, this effect will need to be evaluated on a case-by-case approach to determine which type of vessel is the most appropriate for each formulation being evaluated.

Q **What should be the temperature of dissolution medium during the test?**

A The temperature of the dissolution test is 37 ˚C for products with internal use (see USP general chapters <711> and <1092>) and 32 ˚C for products applied to the skin (see USP <724> and <1724>). For veterinary products, the temperature depends on the animal species (see USP <1236>). Other temperatures may be used with appropriated justification. Examples of dissolution tests approved with other temperatures can be found at https://www.accessdata.fda.gov/scripts/cder/dissolution/ and https://www.usp.org/resources/dissolution-methods-database.

Q **In the USP <711> Dissolution it is stated that if the Stage 1 (S1) dissolution test does not meet the criteria, then the test continues to Stage 2 (S2), and if it still does not meet the test criteria, then continue to Stage 3 (S3). If there is a QC laboratory conducting a dissolution test up to S3 and the results do not meet the criteria, is it necessary to investigate the Out of Specification (OOS) results for the sample or is the dissolution test up to S3 already part of the investigation? If results after S2 already fail S3, is it necessary to perform S3? That is, I already have more than 2 units < Q – 15%. Is it acceptable to state the samples fail S3 without performing S3?**

A The three levels in the acceptance table are part of the results evaluation and are not an OOS investigation. If the sample does not meet the specification at the three levels, the product is considered not meeting the acceptance criteria, and an OOS investigation should be conducted. In this case the purpose of the OOS investigation would be to determine the root cause of the dissolution failure. If you have results at S2 already failing S3, then the results should be discussed within the context of the quality management system at your organization to determine next steps. In some cases, it may be necessary to have additional data obtained from S3, for example, to make the appropriate decision.

Every issue of *Dissolution Technologies* features a Question and Answer section. This section is designed to address general dissolution questions submitted by our readers.

Please send your questions to:
Attn: Q&A
9 Yorkridge Trail, Hockessin, DE 19707
Email: vagray@rcn.com
Submit via our website:
www.dissolutiontech.com

Calendar of Events

November 10, 2022

Dissolution Discussion Group Quarterly Online Meeting— A Trip to the Vet: Expert Advice about Dissolution Testing of Veterinary Products

Location: DDG Online Meeting at 10:30 am ET
Registration: https://www.agilent.com/chem/dissolution-webinars

November 13–16, 2022

Eastern Analytical Symposium and Exhibition

Location: Crowne Plaza Princeton-Conference Center, Plainsboro, NJ, USA
For information, visit eas.org

November 14–17, 2022

GastroPlus Advanced Workshop: DMPK and Clinical Pharmacology

Location: Online
Registration: https://www.simulations-plus.com/events/gastroplus-advanced-workshop-dmpk-and-clinical-pharmacology/

November 16–17, 2022

A Quest for Biowaivers, Including Next Generation Dissolution Characterization and Modeling Workshop

Sponsored by AAPS IVRDT Community and Jagiellonian University

Location: Online
For information, email: quest4bw@uj.edu.pl

November 21–25, 2022

European GastroPlus Introductory Workshop

Location: Online
Registration: https://www.simulations-plus.com/events/gastroplus-introductory-workshop-2/

December 5, 2022

Complimentary Introduction to GastroPlus Workshop

Location: Online
Registration: https://www.simulations-plus.com/events/complimentary-introductory-to-gastroplus-workshop-3

December 7, 2022

IQ Webinar: Dissolution Method troubleshooting - An Industry Perspective

Location: Online, 11 AM EST
Registration: https://attendee.gotowebinar.com/register/5265862876758070541

February 22–24, 2023

Approaches, Regulatory Challenges, and Advances in Bioequivalence, Dissolution Testing, and Biowaivers

Sponsored by AAPS IVRDT Community and College of Pharmacy University of the Philippines Manila

Location: Online from 8 am -12 pm each day, Philippine time (PHT)
For information, email: vagray@rcn.com

February 23, 2023

Dissolution Discussion Group Quarterly Online Meeting— Revisions to USP chapter <1724> Dissolution testing of semisolids

Location: DDG Online Meeting at 10:30 am ET
Registration: https://www.agilent.com/chem/dissolution-webinars

May 25, 2023

Dissolution Discussion Group Quarterly Online Meeting— Looking Ahead: The dissolution lab of the future

Location: DDG Online Meeting at 10:30 am ET
Registration: https://www.agilent.com/chem/dissolution-webinars

July 24–28, 2023

Controlled Release Society 2023 Annual Meeting

Location: Las Vegas, NV, USA
For information, visit http://www.controlledreleasesociety.org/meetings/annual

July 27, 2023

Dissolution Discussion Group Quarterly Online Meeting—Go with your gut: A biorelevant dissolution media discussion

Location: DDG Online Meeting at 10:30 am ET
Registration: https://www.agilent.com/chem/dissolution-webinars

November 23, 2023

Dissolution Discussion Group Quarterly Online Meeting— Dissolution Qualification: The PQ vs MQ debate. What's right for your lab?

Location: DDG Online Meeting at 10:30 am ET
Registration: https://www.agilent.com/chem/dissolution-webinars

Simulations Plus Releases GastroPlus® Version 9.8.3

New update expands the library of virtual populations and enhances connections between software platforms

LANCASTER, CA, October 11, 2022 – Simulations Plus, Inc. (Nasdaq: SLP), a leading provider of modeling and simulation software and services for pharmaceutical safety and efficacy, today announced the release of version 9.8.3 of its flagship physiologically based biopharmaceutics (PBBM) / pharmacokinetics (PBPK) modeling platform, GastroPlus®.

Key enhancements include:

- Improved reporting templates for the Monolix™ software to support the statistical analysis of virtual PBPK population results
- New validated nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH) disease populations with options to inform the NAFLDsym® software
- New validated swine PBPK model to drive pharmaceutical and veterinary medicine research
- New enzyme and transporter expression levels across species to expand the virtual population databases
- Flexible handling of dose regimens to allow for administration of any Additional Dosage Routes model during simulations

Dr. Haiying Zhou, Director of Simulation Technologies said, "Our goals with this release of GastroPlus were twofold: to advance the ways in which our software programs communicate with each other across the Simulations Plus universe and to provide the flexibility our users require to simulate the scenarios they need. We succeeded in this effort through our close collaborations with our partners in industry, academia, and government agencies and by working in harmony with the product development teams of our company."

"We continue to push the boundaries for how PBBM/PBPK modeling should be integrated with machine learning, quantitative systems pharmacology/toxicology (QSP/QST), and population PK/PD approaches," added Daniel O'Connor, director of Business Development. "Our leadership in this space is reflected through the growing number of companies applying GastroPlus to support candidate selection, first-in-human dose selection, formulation optimization, drug-drug interaction assessments, and more – all within a single software environment. We are excited to release this new version to our user community and continue to support and educate researchers worldwide by providing the best and most innovative science."

New Products from Erweka

Langen, October 11, 2022 – ERWEKA GmbH is proud to announce the release of the digital stand-alone Dissolution Offline System for DT 950/9510 Series and the next generation friability and abrasion tester TAR II.

Digital Dissolution Offline System

The new digital dissolution offline system is completely controlled by the digital software platform first introduced with the DT 950. To enable control over the hardware, TestAssist, the dissolution testing assistant has been upgraded to include a new offline testing mode. With this new testing mode, TestAssist is able to control the automated sampling station, connected pump and sample collector for fully automated dissolution offline testing. Furthermore, the ERWEKA operating system running on the DT 950/9510 has been extended with an easy-to-use user management for flexible access control of the full dissolution offline system. The Digital Dissolution Offline System is available for all 6-to-8 test station DT 950 Series and 12-to-14 test station DT 9510 dissolution tester.

TAR II – Advanced friability and abrasion testing with automated calculation and direct balance data connection

TAR II is equipped with a modern touch interface design first introduced with the digital DT 950. It features ERWEKAs testing assistant TestAssist, specifically customized to make friability and abrasion testing as fast and easy as never before. With TestAssist, the TAR II simplifies its testing process and automatically calculates results. Furthermore, a balance can be optionally connected to the TAR II for fully automated weight data transfer during testing with TestAssist.

In the spirit of all recent ERWEKA product releases, TAR II is fully upgradeable even after the purchase – from one to two test stations, and the optional balance connection can also be added later.

Availability, resources, and online demos

Both the Digital Dissolution Offline System and the friability and abrasion tester TAR II are available for order now.

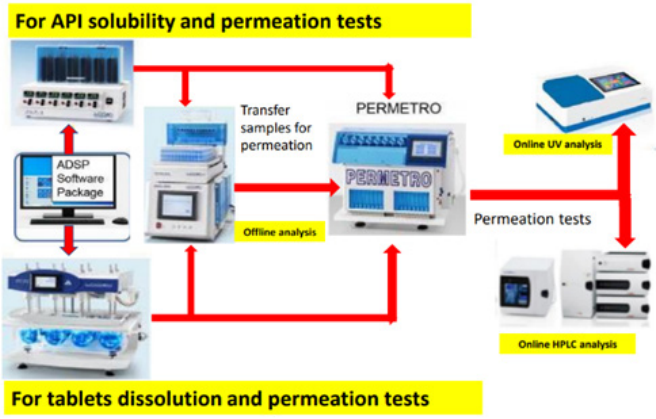
More information such as brochures, technical specifications and videos head over to our website www.erweka.com. We are also happy to show you the Digital Offline System and/or TAR II in an live online demo – just contact your local ERWEKA sales representative or our sales team at sales@erweka.com.

ERWEKA
www.erweka.com

Logan's 3-in-1 Automated Dissolution Permeation (ADSP) System

Logan Instruments is proud to announce its newest innovation is a 3-in-1 system: Automated Dissolution Solubility Permeation (ADSP) system, designed to improve efficiency and accelerate development for pharmaceutical R&D. The ADSP system seamlessly integrates the automated dissolution system, automated solubility testing system, and permeation/absorption system. It has online UV detection and data analytic capabilities. The analysis software runs through the Windows operating system.

The solubility profile for the APIs in different dosage forms is achieved by the Logan STL-100, where the APIs can be determined at six different pH and/or six different temperatures simultaneously. Each solvent and temperature can be changed by the user in six different sample tubes. The system comes with an optional filter system to eliminate undissolved particles. It also provides the accessories to consolidate the API powder to extend the solubility time.



The APIs permeation and/or absorption is tested by using the Logan permeation cells system. This system uses a unique dual cell design, holding an API solution on one side and a receptor solution on the other with a permeation membrane between them. The receptor solution is constantly transferred into a UV spectrum photometer which gives absorbance readout to provide the information of API absorbed by the human body.

After the tablet is formed, the dissolution evaluation is tested using our universal dissolution system. The system contains 8 or 12 vessels that are designed for simultaneous testing for automated dissolution evaluation. The no-vibration and self-calibrating design ensure all tablets dissolve at the speed and temperature the user prefers. A syringe pump and a UV analysis system are attached to the system, designed to analyze the dissolution samples.

The Logan PERMETRO system is used to test the absorption of the tablet in the human body. This system uses a unique dual cell design which holds an API solution on one side and a receptor solution on the other. A permeation membrane is held between the dual cell, and the receptor solution is constantly transferred into the UV spectrum photometer for reading.

The ADSP is the automated all-in-one system designed for pharmaceutical R&D needs in solubility, permeation, and dissolution. The ADSP is in full compliance with 21 CFR part 11 and fully meets GLP requirements. For more information, please contact Logan Instruments.

For more information contact info@loganinstruments.com.

LOGAN INSTRUMENTS CORP.

Agilent Opens Center of Excellence for Dissolution Products

SANTA CLARA, Calif., October 12, 2022 - Agilent Technologies Inc. (NYSE: A) today announced the opening of the Dissolution Center of Excellence (CoE) facility in Craven Arms, United Kingdom. The newly modernized premises will house the core of Agilent's dissolution business, including research and development, quality, support, applications, marketing, and more. This investment allows all Agilent dissolution activities to be consolidated under one roof, facilitating the cooperation of all business segments and driving the mission of innovation for the next generation of dissolution products and services.

The COVID-19 pandemic has highlighted the need for improving virtual connectivity, with many companies seeking new ways to strengthen contact with customers. This expanded site will serve as a hub for Agilent's dissolution customers around the world, providing education, training, application development, and both on-site and virtual demonstrations. These enhanced capabilities demonstrate Agilent's adaptation to this new model of doing business while providing a channel for customers to easily access expertise and support.

Steve O'Donohue, Director at the Craven Arms site, discussed the importance of the new center for Agilent's continuing commitment to dissolution. "The growth in our dissolution business has really driven the need for this larger facility," he said. "Our new flagship site will enable us to develop a truly unified approach to dissolution for the future. The location will also facilitate greater innovation and manufacturing capabilities to support Agilent's digital lab initiatives."

This investment by Agilent also provides immediate value to their global dissolution customer base; Dan Spisak, dissolution Marketing Manager at Agilent, explained. "By centering all dissolution activities in the UK, customers from all time zones will have improved access to our team of experts, with the latest technology to virtually connect for education, training, troubleshooting, and all other types of dissolution testing assistance."

Agilent have a comprehensive portfolio of dissolution testing technology and software meeting pharmaceutical industry requirements. For more information about Agilent's dissolution testing portfolio, visit: agilent.com/en/product/dissolution-testing

About Agilent Technologies

Agilent Technologies Inc. (NYSE: A) is a global leader in the life sciences, diagnostics, and applied chemical markets, delivering insight and innovation that advance the quality of life. Agilent's full range of solutions includes instruments, software, services, and expertise that provide trusted answers to our customers' most challenging questions. The company generated revenue of $6.32 billion in fiscal 2021 and employs 17,000 people worldwide. Information about Agilent is available at www.agilent.com. To receive the latest Agilent news, please subscribe to the Agilent Newsroom. Follow Agilent on LinkedIn, Twitter, and Facebook.

Agilent
Trusted Answers

Copley launches EnviroMate™, an efficient, benchtop environmental chamber for inhaler testing

For the cost-effective reduction in test variability caused by temperature, humidity, and electrostatics

Nottingham, UK, September 14, 2022: EnviroMate™ is a benchtop environmental control chamber from Copley, the world's leading manufacturer and supplier of inhaler testing equipment, designed specifically to improve the repeatability and integrity of inhaler test data. The performance of orally inhaled and nasal drug products (OINDPs) can be directly influenced by ambient temperature, humidity, and electrostatics found in the laboratory, as highlighted by the US and European Pharmacopoeias. EnviroMate is an accurate, efficient, low maintenance solution that addresses these issues with considerable value for scientists faced with:

- Variable lab conditions or inadequate climate control
- OINDPs with high sensitivity to temperature, humidity, and/or electrostatics, such as powders and aqueous formulations where hygroscopicity and evaporation can be an issue
- Poor inter- or intra-lab reproducibility and unexplained out-of-specification (OOS) results
- Achieving better environmental control, in a cost-effective manner, without investing in a dedicated room for testing.

Delivered dose uniformity (DDU) testing and aerodynamic particle size distribution (APSD) measurement by cascade impaction are critical tasks for the inhalation community. Variability in the test environment can affect the dose emission and aerosol generation performance of OINDPs, and APSD measurement methods, compromising the integrity of these tests. By tackling this source of variability EnviroMate reduces the risk of erroneous data and costly testing delays.

EnviroMate is a compact, movable, benchtop unit with a spacious, clear-view chamber, large hinged front door, and slot-sealed rubber entry ports. It comfortably accommodates the Inhaler Testing Workstation™, all types of cascade impactor (including preseparator and mixing inlet as required) with dedicated side wall ports and quick-connectors for interfacing with externally located ancillaries. EnviroMate accurately controls and maintains uniform temperature (± 2°C) and humidity (± 5% RH) by circulating air through the entire chamber, whilst an integrated anti-static system helps to minimise electrostatic effects. The unit contains a self-regenerating desiccant dehumidifier and is refrigerant-free, easing maintenance and environmental impact.

"Controlling environmental conditions precisely during the testing of OINDPs is crucial, but is typically complex and expensive," says Mark Copley, CEO, Copley Scientific. "The EnviroMate is a pragmatic, well-engineered, highly effective option tailored specifically to inhaler testing requirements. It's a great addition to our range and we're sure it will have a positive impact on the quality of test data, for those struggling to achieve stable conditions during DDU and APSD testing."

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The new EnviroMate™ is a cost-efficient, benchtop solution that provides stable environmental conditions for inhaler testing.

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- Ambient to 37°C in less than 15 minutes
- In-shaft continuous temperature sensors
- USP compliant
- Reduced energy usage and operating costs

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Agilent

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Discover Small-Volume Dissolution for Medical Devices

The Agilent 400-DS Apparatus 7 enables dissolution testing of combination products. It is especially ideal for products consisting of a medical device and a regulated drug, such as drug-eluting stents, pacemaker leads, medical contact lenses, and implants.

Low dose? No problem. The 400-DS is suitable for any low-API dosage forms with dissolution tests taking days or more.

Low effort. High throughput. The 400-DS combines dissolution and sampling without the user having to intervene. Plus, up to 13 samples can be simultaneously tested.

Fully compliant. The 400-DS meets USP Apparatus 7 (Reciprocating Disk) requirements, and uses software that facilitates compliance with 21 CFR Part 11.

For more information about the 400-DS, visit:
www.agilent.com/chem/400-ds

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