

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.

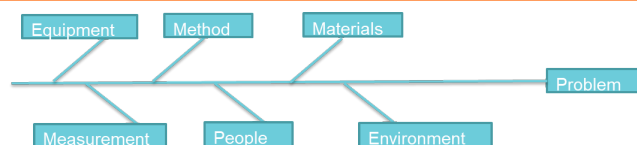


Figure 1. Example of an Ishikawa (fishbone) diagram for dissolution testing.

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.

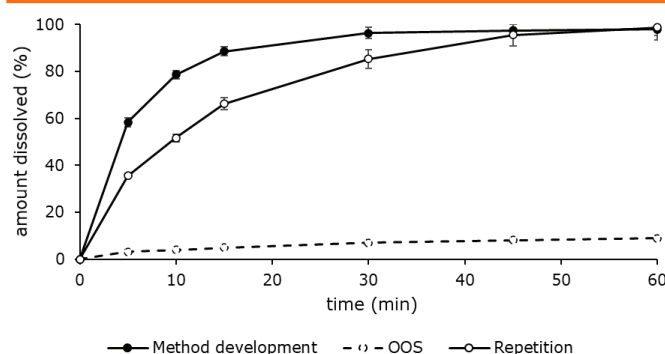


Figure 2. Impact of incorrect buffer medium preparation method on dissolution (case study 1). OOS, out of specification.

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.

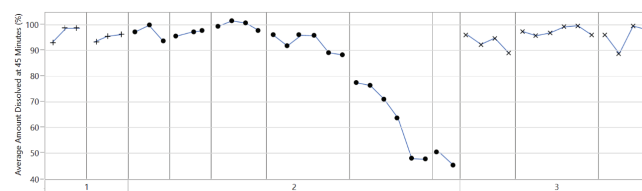


Figure 3. Dissolution release at 45 minutes as a function of media batch and testing order (case study 2).

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.

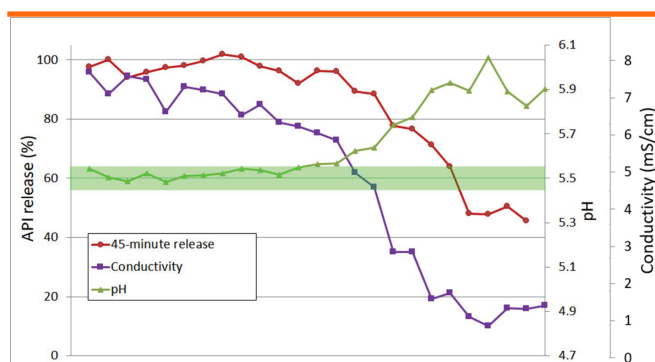


Figure 4. Dissolution at 45 minutes and media pH and conductivity as a function of testing order in inadequately mixed media (case study 2). API, active pharmaceutical ingredient.

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).

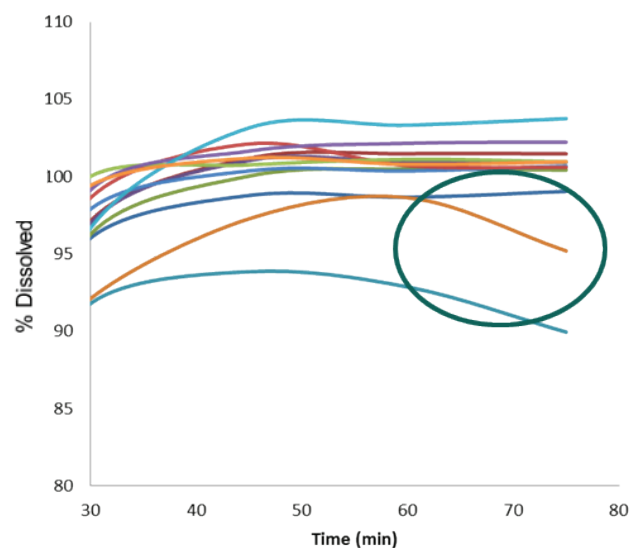


Figure 5. Dissolution curves demonstrating rapid drug degradation in subset of samples from individual vessels (circled) (case study 3).

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.

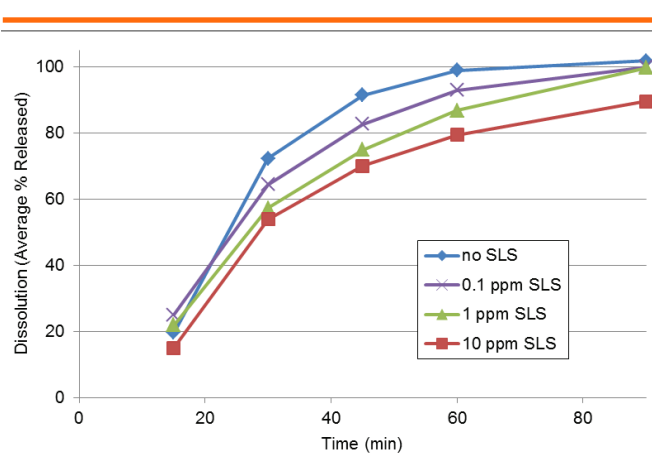


Figure 6. Impact of surfactant contamination of dissolution media (case study 4). SLS, sodium lauryl sulfate.

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).

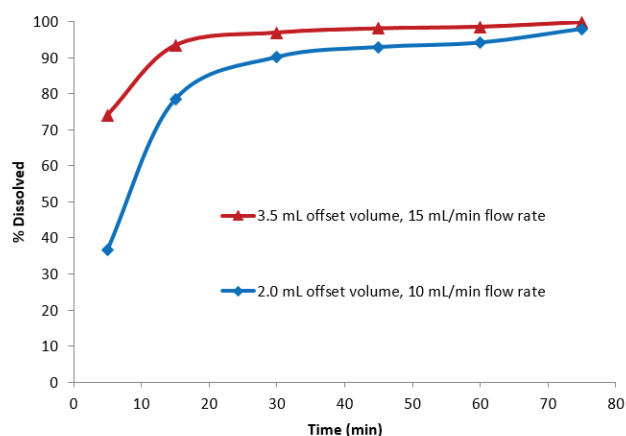


Figure 7. Impact of autosampler method settings on dissolution (case study 5).

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 rate 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

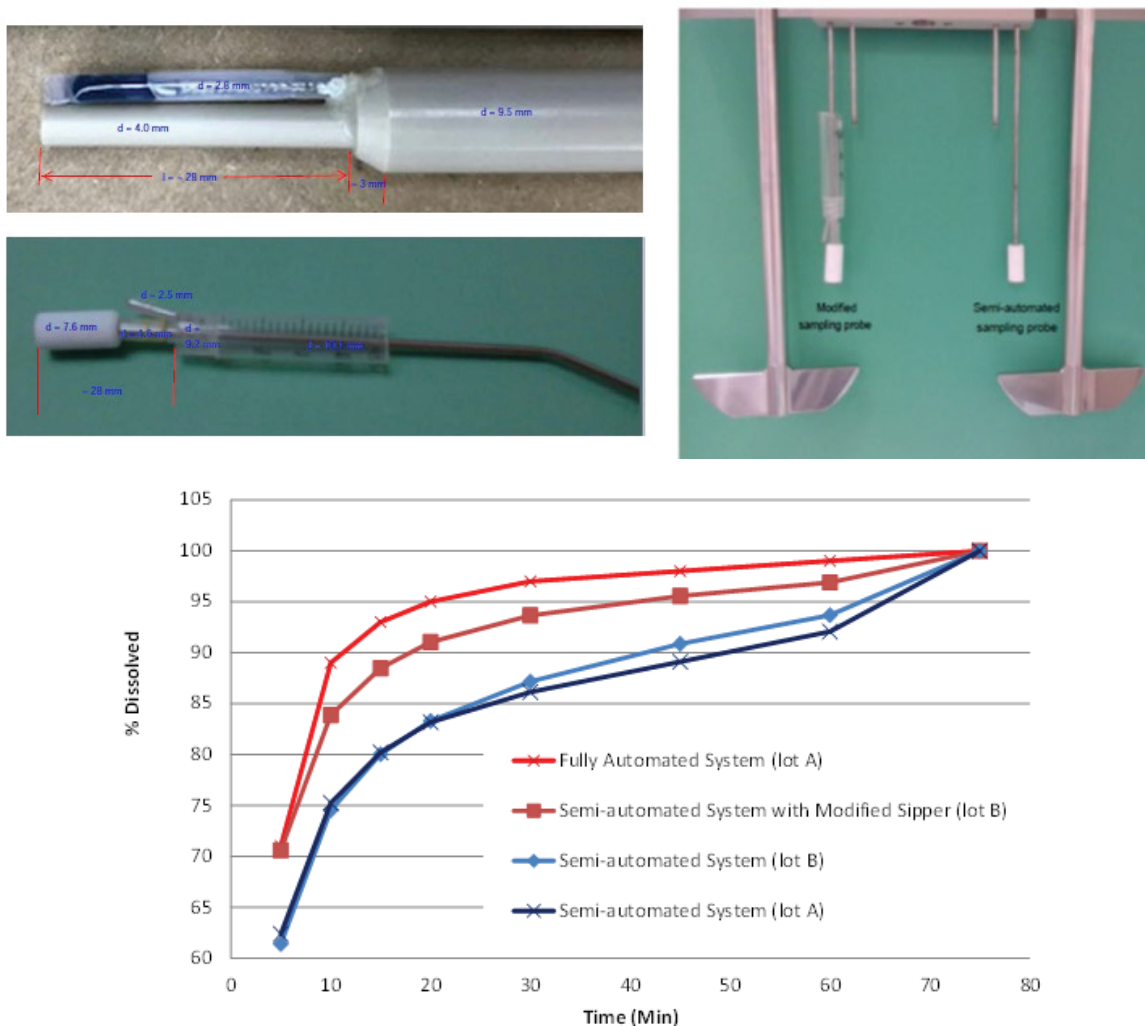


Figure 8. Impact of automated system differences on dissolution (case study 6). Top: Automated sampling probe, semi-automated sampling probe, and probe modified to mimic the fully automated sampling probe. Bottom: Dissolution profiles using USP 2, pH 3.5, 75 rpm through 60 m, followed by an infinity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

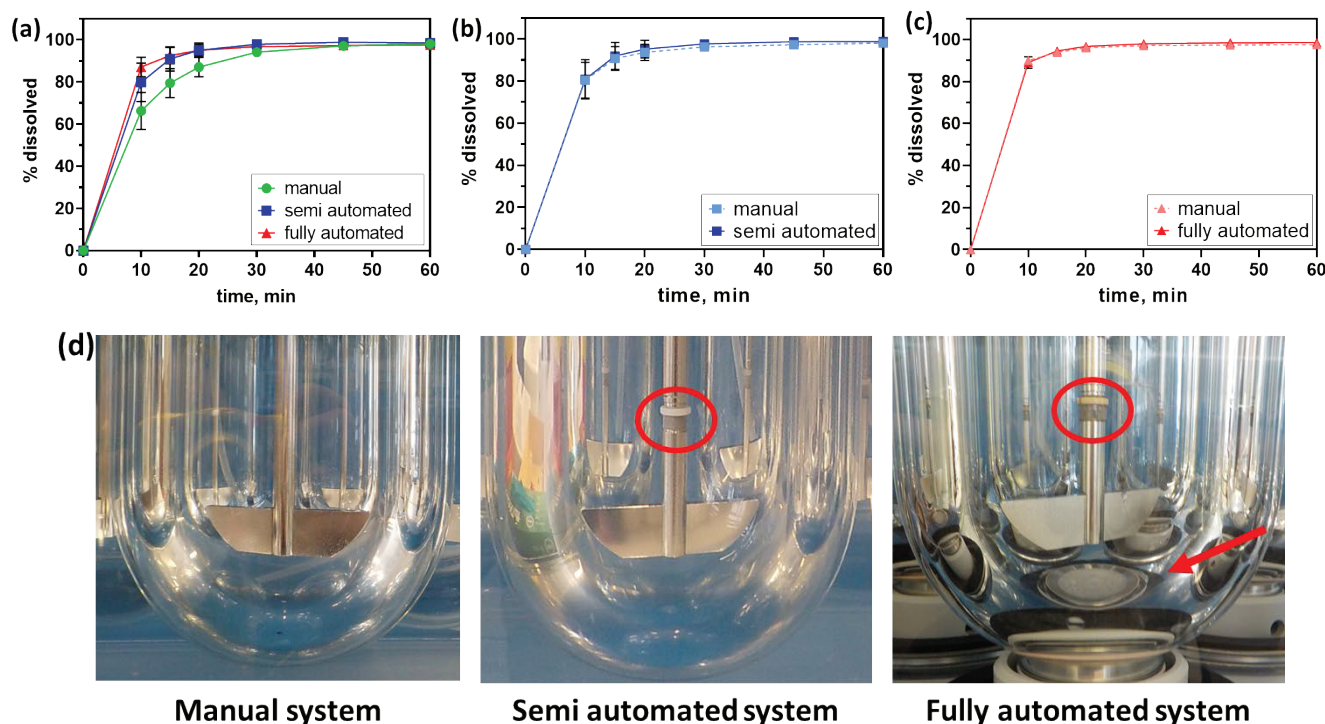


Figure 9. (a) Dissolution profiles using manual, semi-automated, and fully automated setups (case study 7). (b) Comparison between parallel manual and semi-automated sampling. (c) Comparison between parallel manual and fully automated sampling. (d) Vessel setup in each system with marked hollow shaft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

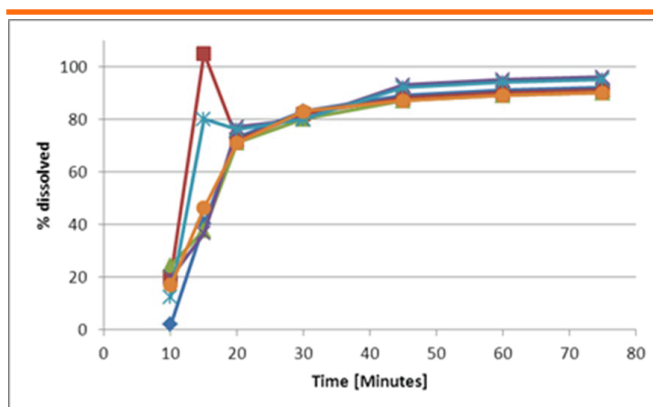


Figure 10. Individual vessel profiles highlighting high percent dissolved values at early timepoints resulting from sampling of undissolved material (case study 8).

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

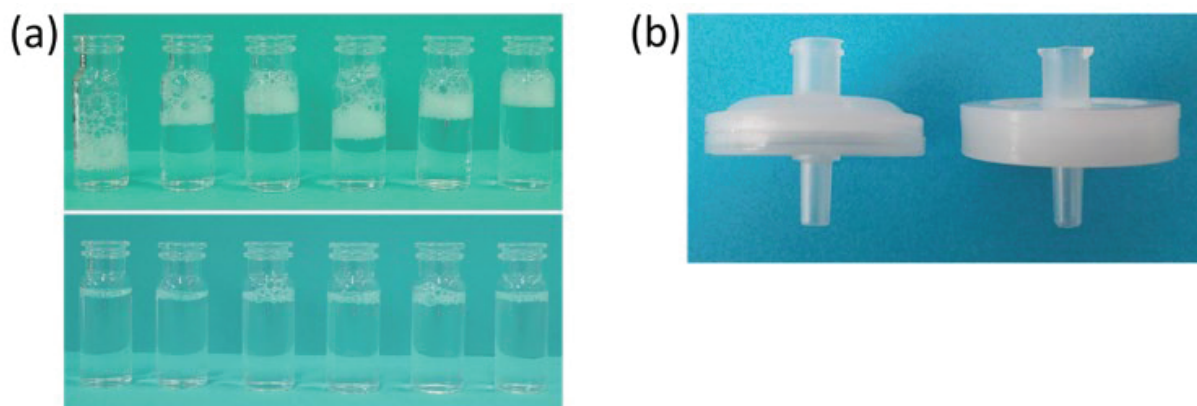


Figure 11. Impact of filter casing geometry on sampling (case study 9). (a) Top row: incomplete sampling due to foaming; bottom row: changing filter type resulted in consistent sampling with almost no foaming. (b) Different casings for the same syringe filter. The square edge Acrodisc filter (right)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

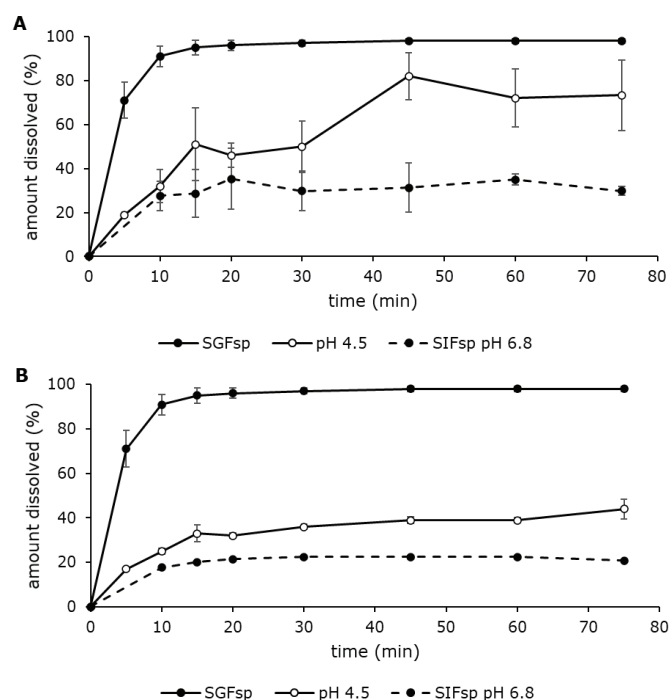


Figure 12. Comparison of dissolution in SGFsp, pH 4.5, and SIFsp pH 6.8 (A) without and (B) with dilution of samples that are prone to post-sampling precipitation (case study 10). SGFsp: simulated gastric fluid without pepsin; SIFsp: simulated intestinal fluid without pancreatin.

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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