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WHITE PAPER

Challenges and Strategies for Solubility Measurements and Dissolution Method Development for Amorphous Solid Dispersion Formulations

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Abstract

This manuscript represents the view of the Dissolution Working Group of the IQ Consortium on the challenges of and recommendations on solubility measurements and development of dissolution methods for immediate release (IR) solid oral dosage forms formulated with amorphous solid dispersions. Nowadays, numerous compounds populate the industrial pipeline as promising drug candidates yet suffer from low aqueous solubility. In the oral drug product development process, solubility along with permeability is a key determinant to assure sufficient drug absorption along the intestinal tract. Formulating the drug candidate as an amorphous solid dispersion (ASD) is one potential option to address this issue. These formulations demonstrate the rapid onset of drug dissolution and can achieve supersaturated concentrations, which poses significant challenges to appropriately characterize solubility and develop quality control dissolution methods. This review strives to categorize the different dissolution and solubility challenges for ASD associated with 3 different topics: (i) definition of solubility and sink conditions for ASD dissolution, (ii) applications and development of non-sink dissolution (according to conventional definition) for ASD formulation screening and QC method development, and (iii) the advantages and disadvantages of using dissolution in detecting crystallinity in ASD formulations. Related to these challenges, successful examples of dissolution experiments in the context of control strategies are shared and may lead as an example for scientific consensus concerning dissolution testing of ASD.

Keywords Dissolution · Amorphous solid dispersions · Solubility · Crystallization

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Introduction

Since a growing proportion of compounds entering drug development can be characterized as poorly soluble (1), enabling in vivo absorption through improving the drug's apparent solubility and dissolution rate in the human GI tract has been one of the major challenges facing pharmaceutical development scientists. Creation of amorphous solid dispersions (ASDs), that is, a mechanism for manufacturing and retaining a drug substance in an amorphous form by dispersing with a polymer that retards or prevents crystallization, is a commonly used formulation approach to address this challenge. The advantage of amorphous dispersions is realized in the higher kinetic solubility over the crystalline form of the drug leading to the possibility to form supersaturated solutions upon dissolution of the ASD. This behavior can help in overcoming the solubility limitation of some conventional formulations. Lyophilization, spray

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drying, hot-melt-extrusion, and cryo-milling are just a few examples of technologies that are used widely for creating amorphous APIs or drug product intermediates in formulation development to improve the apparent API solubility and dissolution rate (2).

The advancement of amorphous formulation technologies in recent years comes along with new challenges to the development of dissolution methodology which can range from appropriate biorelevant dissolution tests for formulation screening to QC method selection and product specification setting. In particular, the IQ Consortium surveyed on this topic in 2017, which is summarized in this paper. The survey found that most of the 21 companies that responded agreed that they commonly face dissolution-related challenges during drug development. Some of the challenges were attributed to increased regulatory expectations on the discriminating ability of a dissolution method. Other challenges were more technical such as the ability of a dissolution method to detect crystalline API or the ability to measure drug solubility that is representative of the respective ASD formulation. Thus, the dissolution challenges are categorized into three types in this paper: the definition of solubility and sink conditions for ASD dissolution, challenges in the development of non-sink dissolution (according to conventional definition) for ASD formulation screening and QC methods, and the advantages and disadvantages of using dissolution in detecting crystallinity of ASD formulations. In addition to describing these challenges and scientific approaches, some successful examples of utilization of dissolution for control strategies for ASD formulations are also discussed in this paper. These are presented to stimulate the sharing of more information within the pharmaceutical development community to further align and advance scientific principles for ASD dissolution.

Solubility Measurements for Defining Sink Conditions and QC Dissolution Method Development

Processes in the Dissolution of ASD Formulations (and How Solubility Measurements Can Help in Their Understanding)

Poorly water-soluble drugs face two major challenges: First, they often dissolve very slowly; hence, the dissolution rate may be too slow for adequate absorption and subsequently limit bioavailability (dissolution-rate limited). Second, assuming that only molecularly dissolved drug passes the enterocytes, the low solubility of the drug in the intestinal fluids may lead to solubility-limited drug performance. Enabling formulation strategies such as ASDs address these obstacles by improving apparent drug solubility through supersaturation and dissolution rate/extent in the GI fluids. In general, a metastable supersaturated state of the respective drug is induced and precipitation is potentially inhibited, finally aiming to improve overall drug absorption and bioavailability (1, 3).

Dissolution of ASDs

Dissolution is a multi-step process involving the disintegration or erosion of a pharmaceutical formulation, followed by solubilization of the API. The first step is influenced by the choice of excipients such as polymers and/or disintegrants used in the formulation. The second step involves the interaction of the API with the surrounding medium. Upon contact with an aqueous medium, e.g., dissolution medium or GI fluid, the solid ASD formulation may absorb water and start to swell. Polymers, used for dispersing the amorphous form of an API in the formulation, can prevent amorphous-amorphous phase separation and consequently should inhibit the crystallization of the API (4, 5). Ideally, an amorphously embedded API dissolves from an ASD into the aqueous medium until the full dose dissolves or reaches its amorphous solubility limit. At the same time, water will penetrate the amorphous drug matrix, which, in turn, leads to saturation with water. Consequently, a metastable equilibrium between these two phases is established (6). Due to the higher Gibbs free energy in the ASD, the API concentration in the aqueous phase can increase rapidly and a supersaturated solution, relative to the solubility of the most stable crystalline form, is achieved. Microenvironments, such as those created with bile salts or mixed micelles of lecithin and bile salts as well as matrix polymers, may help to stabilize the supersaturation via interaction with the API molecules (7), but have also been observed to promote crystallization of the API (8). Since these microenvironments are thermodynamically unstable, API crystallization can occur in either the hydrated ASD matrix or the aqueous phase. A recent study by Moseson *et al*. (3) found that in fully amorphous ASDs, nucleation was initiated at the solid/water interface and then proceeded from there throughout the solid. In contrast, for ASD samples containing residual crystallinity, crystal growth started within the sample. Dissolution of the samples then resulted in a locally high supersaturation in the amorphous solid surrounding the crystals, which caused additional crystal growth resulting in larger crystals and/or agglomerates. The two different crystallization processes in the solid ASDs continued both until the entire amorphous content was crystallized, which, in turn, resulted in the termination of the dissolution process in case of a closed dissolution system. As a consequence, crystallization resulted in rapid depletion of the supersaturation, which reduced the driving force for drug flux across the intestinal membrane, drug absorption, and, finally, the loss

of the ASD benefit (9, 10). Especially at high drug loading (e.g., 40–50%), there is a potential risk of having no simultaneous release of polymer and API resulting in a fast onset of crystallization and precipitation along the intestinal tract. The use of a surfactant has shown to have a beneficial effect on the congruent release of API and polymer to reach for the liquid–liquid phase separation (LLPS; see below), resulting in a metastable state of supersaturation (11), but have also been observed to promote crystallization and limit the effectiveness of polymers on inhibiting crystallization (12).

Supersaturation and Liquid–Liquid Phase Separation (LLPS) Formation

ASDs typically dissolve rapidly from the formulation and form a maintained supersaturated state. If the volume of the dissolution medium is equal to that required to dissolve the solid in its amorphous state, the ASD may dissolve and then precipitate in two different patterns. Firstly, for high solubility ASDs with low drug loading, the dissolution is mainly polymer-controlled and complete dissolution can occur (13). The degree to which a solution is supersaturated affects different kinetic processes such as nucleation and crystal growth, as well as solute diffusion across a membrane that is lowered in case of drug precipitation. The supersaturated state aims to stabilize an intermediate phase with drug concentrations above the kinetic solubility where drug-rich nanodroplets (liquid–liquid phase separation, LLPS) are formed. These droplets can act as reservoirs for the supersaturated state but can also act as a precursor to crystallization (10) (Fig. 1). LLPS formation is dependent on multiple factors such as drug loading, the characteristics of the polymer of the ASD formulation, tendencies of the drug to crystallize, the particle size of the dispersion, and medium composition and volume. Secondly, for formulations with high drug loading not exceeding the kinetic solubility, as well as for rapidly crystallizing drugs, no LLPS is observed (14).

From a pharmacokinetic (PK) perspective, passive drug absorption can be influenced by the extent and duration of the supersaturated state of a drug in the fluid contents of the GI tract (15). In cases of high permeability compounds, the flux of free drug increases linearly until the upper limit of supersaturation is achieved, which is defined by the liquid–liquid phase transition concentration ($C_{L\text{-}L}$ boundary) (10). By reaching the amorphous solubility limit of the compound, the flux reaches a maximum value. Further increase in drug concentration does not additionally increase the transport across the membrane (16). Whereas the supersaturated state can be influenced by polymers and surfactants in the ASD matrix and dissolution medium, their influence on the permeability of the drug is not always positive and has to be investigated on a case-by-case basis (17).

Solubility Measurements as a Basis for Dissolution Method Development

QC dissolution methods are developed to reflect the properties of the dosage form and to indicate potential changes in drug product performance, e.g., deriving from changes in quantitative composition, critical material attributes (CMAs), or critical process parameters (CPPs) during manufacturing. As stated in USP < 1092 > (18), if sink conditions are present, the dissolution rate is for example limited by the API particle size and formulation properties rather than the API equilibrium solubility. For this purpose, the pH, composition, and volume of the dissolution medium are chosen to have at least three times (USP < 1092 >) or 3 to 10 times (Ph.

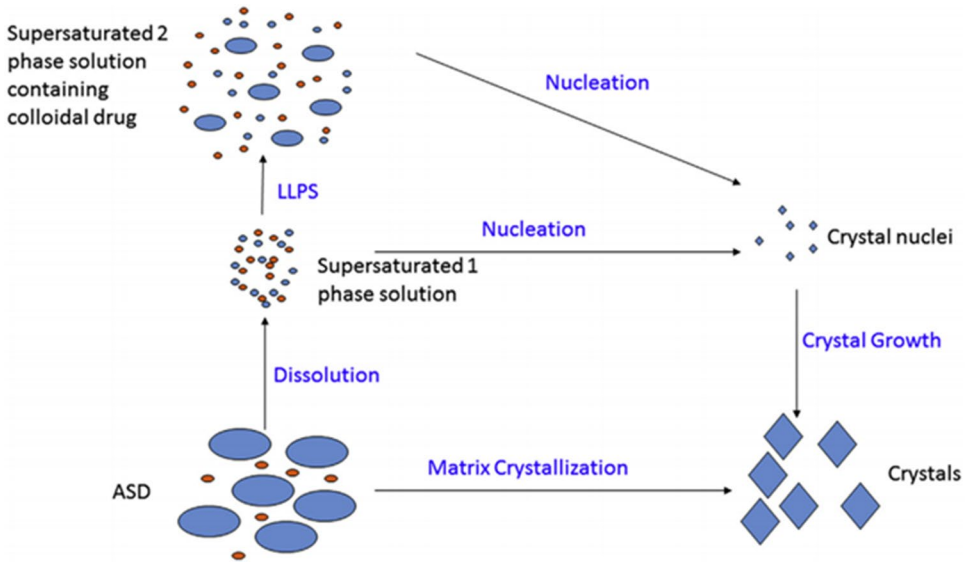

Fig. 1 Schematic illustrating the different possible crystallization routes during the dissolution of an amorphous solid dispersion under non-sink conditions. Printed with permission of Elsevier (10)

Eur, (19)) the volume required to form a saturated solution and thus achieve sink conditions.

However, ASDs are highly complex formulations that introduce a multitude of additional parameters that are not reflected in the traditional solubility measurements of the crystalline drug. As described previously, this formulation approach relies on stabilizing the amorphous state of a drug and introduces additional microenvironments upon drug dissolution. Their characterization and understanding can substantially help to guide formulation development and to choose better conditions and media for dissolution method development. In order to align on general terminology, some frequently used terms with regard to dissolution and solubility of ASD formulations are summarized in Table I.

Thermodynamic Solubility

Solubility measurements are routinely performed at various stages throughout the development process and have a critical impact on drug classification and formulation design (20). The proper dissolution medium for QC measurements is typically selected after conducting solubility measurements in various potential media spanning the physiological pH range (1 to 6.8 or 7.5). If necessary, this includes potential surfactants and is done via the shake flask method at 37°C to determine the saturation concentration of the drug, mostly referred to as the equilibrium solubility of the most stable form under these specific conditions (18).

Although this approach may seem straightforward, all parameters involved in the measurement are highly sensitive to deviations if not properly defined as recently found by a study across seven pharmaceutical companies in Japan (Consortium of Biopharmaceutical Tools, CoBiTo) (21). Harmonizing experimental procedures resulted overall in more comparable solubilities and significantly lowered inter-laboratory variability. New APIs are usually characterized in different labs and possibly across multiple sites within the same company. Therefore, clearly defined parameters for solubility measurements may help ensure robust and comparable results.

Many companies and organizations such as the United States Pharmacopeia (22), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (23), and the World Health Organization (24) already have a protocol for thermodynamic solubility measurements; to ensure best results, companies may consider following recommendations as discussed by Avdeef *et al.* (25). Agitation of the drug particles should be sufficient to firmly wet and suspend them, e.g., by stirring or shaking. Strong forces such as ultrasonication, however, should be avoided as they may increase the tendency of the compound to agglomerate. The dissolution experimental temperature of 37°C should be also used for solubility measurements (23). Measurements at other temperatures, e.g., 25°C, can affect the results significantly (21). Additionally, samples need sufficient time to achieve equilibrium, which is usually reached after 24 to 48 h and the correct measurements of samples with low solubility can be challenging. Moreover, sampling at intervals before reaching the equilibrium can give valuable insights into the kinetics of the drug solubility and indicate the time when the final plateau is reached. In terms of sample preparation, filtration should be favored over single centrifugation as the latter may result in higher measured solubilities due to insufficient separation of dissolved from the undissolved drug as illustrated in the CoBiTo study (21). Filter materials should be selected based on the knowledge of the affinity of the drug to adsorb to them and, like pipettes, should be pre-wetted prior to use.

Especially in the early stages, only small quantities of the new compound are available, which often vary in their quality due to higher levels of impurities and small batch sizes

Table I Definitions of Frequently Used Terms Concerning QC Dissolution Method Development and ASD Dissolution Testing

Term	Definition
Kinetic solubility	The concentration of dissolved drug before equilibrium concentration is reached. In the context of ASDs, this is often referred to as "amorphous or apparent solubility"
Amorphous solubility limit	The maximum limit to which amorphous drug material will dissolve right before liquid–liquid phase (LLPS) separation may be observed. Typically, above 100% dissolution limit for crystalline form
Thermodynamic solubility	The concentration of dissolved drug once equilibrium concentration is reached. In the context of ASDs, this is often referred to as "crystalline solubility"
Crystalline solubility limit	Maximum limit to which crystalline material may dissolve. Above this limit, only amorphous drug material will dissolve
Sink conditions	Dissolution conditions, i.e., pH, medium composition, and volume. Dissolution medium volume that ensures at least three times (USP 1092) or 3–10 times (Ph. Eur. 5.17.1.) the volume required to form a saturated solution. Considerations are commonly based on thermodynamic solubility data
Non-sink conditions	Dissolution conditions that are failing to provide sink conditions based on commonly applied thermodynamic solubility considerations

being produced. Thus, solubility measurements may have to be scaled down to allow a comprehensive and economical characterization such that test tubes or even multi-well plates may be used in the experimental setup. As small variations can influence the measured solubility significantly, a comprehensive experimental protocol for small-scale experiments is even more important (20).

For all solubility and precipitation measurements presented in this paper, online UV measurements can help to face these challenges as the experimental steps are kept to a minimum. By using UV probes with interchangeable pathlengths in the tip, small volumes with 10–100-µg material in a 1–20-ml medium can be analyzed since samples do not have to be withdrawn, thus maintaining the volume at a constant level (26, 27). As an alternative, inline UV measurements using flow-through cuvettes can also be used to collect data over multiple time points during an experiment (28). Both techniques do not require sample preparation and can be automated. Data are collected constantly during the experiment, which allows for inline monitoring of solubility processes.

A high and/or variable impurity content of the drug substance can impact the solubility by causing interferences with in situ readings as well as with classical off-line techniques such as manual sampling followed by HPLC analytics, e.g., due to self-buffering or solid-phase conversions affecting the surface and/or bulk pH. Hence, it is important to monitor the pH before and after the experiment in case the buffer capacity of the selected medium is insufficient. Additionally, it is recommended to further investigate the remaining solids and/or precipitate using XRPD, FTIR, TGA, DSC, or hot-stage microscopy. These techniques can be used to detect and identify processes like conversions from salt to free form or the crystallization to a different polymorph with as little as one crystal needed (29).

It may be helpful to update initially measured solubilities during development due to changed API qualities, improved formulations, or new dosage strengths. At later development stages, when a sufficient amount of the final API quality is available, solubility measurements should be re-determined using larger API amounts and media volumes using a standardized procedure. These final solubility measurements can then be used in the confirmation of drug classification and dissolution method justification concerning the API present in the future (marketed) product.

Kinetic Solubility and Supersaturation

Ideally, the entire quantity of API within the solid formulation is fully amorphous. When measuring solubility for ASDs, one should measure the kinetic ("amorphous") solubility, rather than the thermodynamic (crystalline) solubility. Furthermore, the kinetic solubility represents a measure of the supersaturation limit of a formulation. However, depending on the compound characteristics, reliable kinetic solubility measurements can be challenging. For slowly crystallizing compounds, simple setups for kinetic solubility measurements mimic the shake flask method that is typically used for routine solubility measurements of crystalline API. Amorphous material can be prepared by melt quenching, spray drying, or spin coating. Similar to thermodynamic solubility measurements, the amorphous API is agitated in the selected aqueous medium and the concentration is monitored over time until an equilibrium/plateau is reached (17, 30).

"Fast crystallizers," however, can start to crystallize upon contact with an aqueous phase, or in some cases, even in environments of slightly increased humidity. An alternative approach to the shake flask method is the solvent shift method, where a supersaturated solution is created by dissolving the crystalline drug in an organic solvent such as methanol, DMSO, or THF (31). The drug/organic solvent mixture is subsequently added to the aqueous medium and precipitation is monitored via UV spectroscopy (Fig. 2). To limit the effect of the organic solvent on the aqueous phase as much as possible, the concentration of the drug in the organic solvent should be high enough so that no more than a total of 1% API solution dissolved in an organic solvent has to be added to the aqueous phase (32, 33). To stabilize the supersaturated state, polymers may be added to prevent nucleation.

As an alternative approach, as suggested by some answers to the 2017 survey, the ASD drug product intermediate, e.g.,

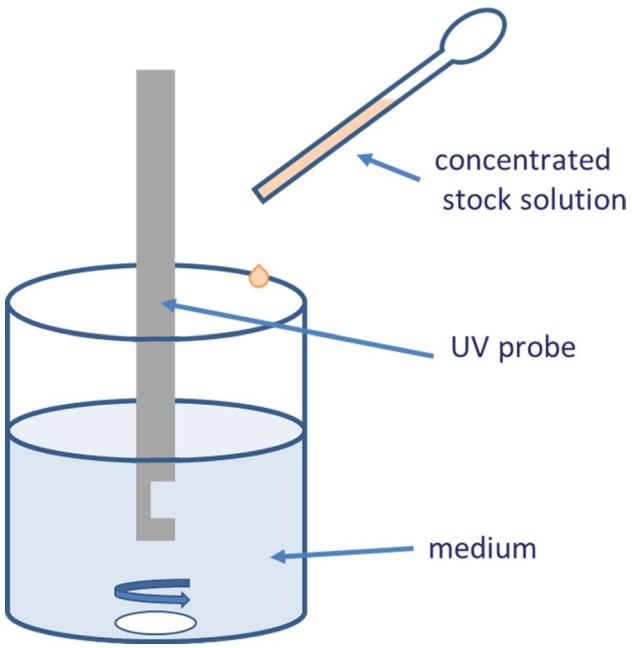

Fig. 2 Setup of the solvent shift assay to measure kinetic drug solubility. Adopted and modified from Plum *et al.* (32)

the hot-melt-extrudate, may be used directly in solubility measurements and the level of supersaturation achieved from the ASD can be measured. However, both presented approaches to measuring the kinetic solubility have some drawbacks and may not capture the actual solubility of the amorphous drug, but only a value close to it. Specifically, by utilizing the latter approach the actual kinetic solubility might not be reached due to the presence of equilibrium of dissolution of amorphous material and precipitation. Compared to thermodynamic solubility measurements, determining the kinetic solubility is even more prone to inconsistencies. If this is an issue, both approaches along with different experimental setups (e.g., pH shift) should be investigated when possible (31).

All formulation advantages of ASDs result in processes, e.g., the fast release of an amorphous drug, which is stabilized in a supersaturated state and cannot be measured adequately by single/endpoint measurements. Kinetic profiles of crystalline and amorphous drug capture the rate of API solubilization and potentially the ability to supersaturate, before accurately determining the equilibrium solubility. The solubility measurements presented here can provide valuable insights into dissolution processes and may guide formulation and dissolution method development as well as improve the understanding of processes observed during dissolution.

In this paper, we will refer to thermodynamic sink conditions which are established based on USP < 1092 > (18) with the thermodynamic (crystalline) solubility of the API. In contrast, non-sink or kinetic sink refers to conditions in which the thermodynamic (crystalline) solubility in a certain dissolution medium is less than three times the concentration in the medium. However, based on the kinetic solubility, sink conditions might still be met. As every ASD formulation has its challenges, characterization techniques may also vary and will have to be adjusted. For something as simple as a "basic" solubility measurement, standardized protocols help to create robust data to rely on in later stage development.

Precipitation and Dissolution Evaluation of ASD Formulations During Drug Development

ASD formulations are developed specifically to stabilize an amorphous drug within a solid pharmaceutical formulation and ideally, also during dissolution in an aqueous medium, to achieve a prolonged supersaturated state often referred to as the "spring and parachute" approach (Fig. 3) (34). As these are intentionally developed characteristics of the solid dosage form, evaluation of the ability of a formulation to achieve supersaturation, and possibly maintain it, provides valuable additional insight into the functionality and quality of the formulation during development. To more closely examine supersaturation and precipitation behavior as well as the risk of in vivo precipitation of a poorly soluble drug,

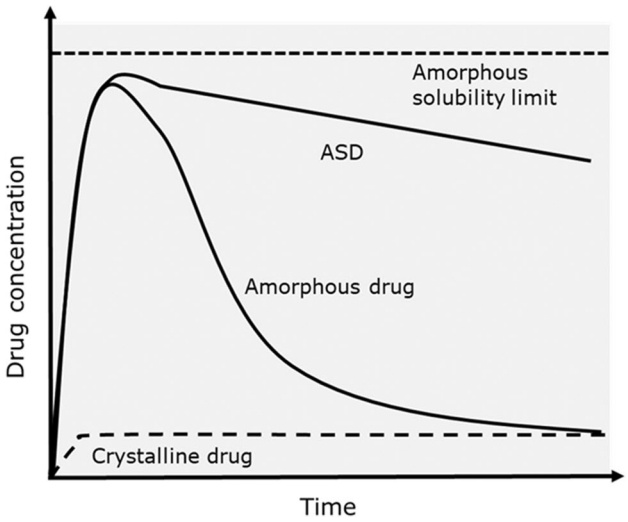

Fig. 3 Schematic representation of kinetic/thermodynamic solubility behavior of crystalline vs. amorphous drug vs. drug formulated as ASD formulation and amorphous solubility limit

pH shift or solvent shift methods can be used. By removing the dissolution step, the precipitation in biorelevant media, such as FaSSIF, can be closely examined. The experiments are commonly conducted in a miniaturized setup. Since the supersaturated state is highly unstable, it cannot be captured with a single-point measurement but must be assessed over time. Monitoring is often done via inline measurements with UV probes as described in an interlaboratory variability study during the OrBiTo project to establish robust protocols for supersaturation and precipitation measurements (26). Unfortunately, even with standardized protocols, precipitation is highly unpredictable and lacked repeatability. However, this approach may be used to rank formulations qualitatively (31) by evaluating how long a solution can stay supersaturated before precipitation occurs as a function of varying concentrations. Additionally, these solvent and pH shift methods can be used to investigate the effectiveness of various polymers in the formulation in helping maintain the supersaturated state (35) making them a valuable tool in formulation development. This effectiveness in precipitation inhibition can be directly expressed as the excipient gain factor (36).

Due to the impact of experimental conditions on the precipitation kinetics, it may be of interest to monitor the polymer concentrations in addition to the drug concentrations during the dissolution experiment, especially in the view of high drug loading ASD formulations to assure the congruent API-polymer release. If, in this case, a non-congruent release will be observed, the risk of creating an amorphous-amorphous phase separation may occur with a risk of minimal drug release (37).

Various dissolution approaches are commonly used during drug development to examine the performance of ASD formulations (1, 6). During drug development, dissolution tests are often performed under pre-defined "bio-relevant" conditions by dissolving the dosage form in a single dissolution medium, often physiologically relevant one (i.e., Simulated Gastric fluid (SGF) or Fasted State Intestinal Fluid (FaSSIF)) or a combination of both in a 2-stage dissolution experiment aiming for simulating the gastrointestinal transfer of the drug/drug product (30, 31). Measurements using media such as FaSSIF may be helpful to assess the effect of bile salts on the ASD formulation (38). These types of measurements may be automated to a certain extent, e.g., by using inline UV measurements, as studied during an inter-laboratory variability study during the OrBiTo project with 12 labs (26). For formulations containing amorphous solid dispersions, these conditions are often non-sink conditions concerning the thermodynamic solubility of the drug due to using physiologically relevant fluid volumes resulting in supersaturated solutions formed upon the dissolution of the ASD.

While these experiments mentioned here are well suited to characterize dissolution and precipitation behavior, single vessel dissolution experiments might have a limited capability of predicting in vivo precipitation risk of ASD formulations. In general, precipitation from supersaturated systems is strongly dependent on the medium composition, medium pH, hydrodynamics in the vessel, and concentration which is often different for human physiology when compared to conditions used in these experiments. Additionally, absorption of the drug in vivo will actively lower the drug concentration resulting in a relatively lower precipitation risk. To more closely mimic the in vivo conditions, non-standardized dissolution systems are frequently employed during development to inform about their biopharmaceutical risk of the formulation (39). In order of increasing complexity, these systems include the use of biorelevant media, use of pH shift testing, and/or use of multi-compartments such as artificial stomach duodenum (ASD) model (40), BioGIT (41), Gastrointestinal Simulator (42, 43), GastroDuo (44), and TIM-1 (45). Most of these advanced dissolution setups take into consideration more gradual transfer from gastric conditions to intestinal conditions, biorelevant media, and volumes, and often employ even a mechanism of removal of the dissolved drug, mimicking drug absorption. These dissolution systems are specifically well suited for possibly predicting the in vivo dissolution and precipitation risks of ASD formulations (41, 46) and for rank-ordering formulation performance during development. While multicompartmental systems are widely used for development purposes, these dissolution systems are of limited utility in a QC environment due to a lack of standardization and robustness coupled with their high complexity (47) which can make their use very labor-intensive.

Is Sink Condition as Classically Defined a Viable Parameter to Establish a Discriminative QC Dissolution Method for ASD Formulations — Considerations for Method Development

Current regulatory guidelines do not address appropriately how to develop dissolution methods for product release testing in a quality control (QC) environment for ASDs. Sink conditions in QC dissolution method development are typically discussed relative to the thermodynamic equilibrium solubility and measured according to USP < 1092 > (18). The mere discussion of dissolution methodology in terms of sink conditions relative to the thermodynamic solubility, referring to the crystalline drug, in ASD formulations does not address the highly complex nature of the system and may result in incomplete and/or wrong interpretation of the measured dissolution data. Also, the term "non-sink" is neither standardized nor clearly defined (e.g., not mentioned in USP or Ph. Eur.). When referring to non-sink conditions, it would be helpful to clarify what the term is referring to in each case. From a USP perspective, sink conditions are not required for QC dissolution method development if appropriately justified. To identify sink conditions, solubility traditionally refers to the pure crystalline API.

In the 2017 IQ survey, over 70% of the respondents replied that they do not (solely) rely on solubility measurements of the crystalline drug to define solubility or sink conditions. The kinetic solubility, capturing amorphous drug performance, is used to set sink conditions, guide medium selection, or aid QC dissolution method justification. Due to the variety of different approaches used by the responding companies, most of them seem to have implemented their strategy to set dissolution parameters for ASD formulations.

For this purpose, the application of the sink index (10) can be helpful in defining sink conditions more clearly. The sink index is the ratio of the thermodynamic solubility of the drug over the dose divided by the dissolution volume.

Under pure non-sink or supersaturated conditions (sink index < 1), method discrimination for ASD formulations is driven by formulation and API; hence, using one of these conditions might be ideal to establish a QC method. However, as there are at least two different API states, it is important to mention which solubility is the basis for defining sink conditions. For example, kinetic solubility values of an amorphous drug are substantially higher than thermodynamic solubility values. Hence, a dissolution method may be "non-sink" pertaining to the thermodynamic solubility but "sink" concerning the kinetic solubility. Moreover, the definition of a factor based on kinetic solubility would be helpful to allow for a mechanistic and consistent dissolution method development of such formulations. Based on these considerations, when selecting the appropriate dissolution medium for a QC method for ASDs, it is essential to

measure and understand both the thermodynamic solubility as well as the amorphous solubility in the dissolution media. Since the drug is present in its amorphous form in the drug product, it is reasonable to use the kinetic solubility values as guidance for media selection. With commonly reported amorphous solubilities from twofold to 35-fold (48, 49) higher than the thermodynamic solubilities, dissolution media selection based on kinetic solubility should have the capability to be discriminating towards the presence of the crystalline drug in the formulation, if the ratio of dose/volume is lower than the amorphous solubility but higher than the thermodynamic solubility (i.e., sink index < 1). Detectability of crystalline material in such methods might manifest in a lower extent of drug dissolved during the dissolution test due to the low solubility of the crystalline material in the media. This can be seen in later time points on the dissolution curve when a plateau (e.g., 60 min for immediate-release tablets) is reached (50). The general schematic of the detection of crystalline material in a QC dissolution test is illustrated in Fig. 4.

However, non-sink dissolution conditions with sink index < 1, which show more discriminating ability, might be hard to implement in a QC environment due to possible robustness concerns; potential crystallization of dissolved drug during the dissolution experiment or between sampling and off-line analysis of the dissolution sample could result in inaccurate results. This "in-solution crystallization" can severely limit the sample stability during the dissolution experiment which might limit the applicability in QC laboratories.

This should be taken into consideration when selecting media pH, volume, and surfactant type and concentration along with careful evaluation of the dissolution equipment to ensure that no heterogeneous nucleation sites such as scratches in dissolution vessels are present.

Real-time in-vessel fiber optic detection techniques, as well as automated flow-through UV analysis, are ways to overcome these limitations of short sample stability; however, this is typically not employed in QC-type settings. Alternatively, samples can be stabilized post-sampling via a dilution with a strong (organic) solvent to increase the solubility of the supersaturated drug in the sample vial. However, subsequent sample dilution adds additional steps to the execution of the test and will increase the complexity of the test and possibly add further variability to the results.

In addition to these considerations, various other effects like slow tablet disintegration, tablet matrix effects, and dissolution artifacts, such as coning, wettability, crystalline particle size (50) etc., can affect the dissolution rate and extent of dissolution. While conceptually, QC dissolution methods can be discriminating towards potential crystallization in ASD formulations, the capability to utilize them quantitatively might be limited and should be evaluated on a case-by-case basis.

In addition to the detectability of potential form conversion, the discriminating ability of the dissolution method towards other critical material attributes (CMAs) and critical process parameters (CPPs) should be evaluated and optimized as part of method development to develop a suitable QC dissolution method.

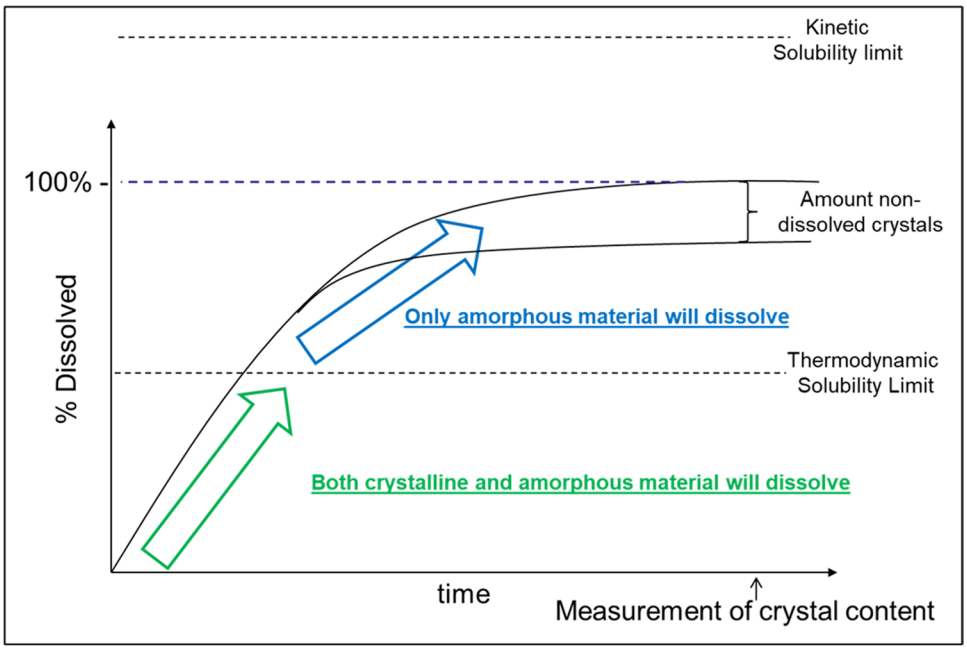

Fig. 4 Ideal dissolution behavior for amorphous solid dispersions to discriminate between crystalline and amorphous drug reprinted from (50)

Crystallinity Detection: How QC Dissolution Is Used to Detect Crystallinity in QC Environment

For a drug product made with ASD, physical form is typically a critical quality attribute (CQA); therefore, detection and control of crystallinity are often required. By application of ICH Q6a Decision Tree 4 (51), there are three broad scenarios for setting acceptance criteria for the CQA of crystalline content in an ASD formulation. These are:

1. A form control is required, and the control strategy employs indirect determination of crystalline content, using dissolution testing as a surrogate for polymorphic form.
2. A form control is required, and the control strategy employs the direct measurement of crystalline content, using a technique such as XRPD, DSC, microscopy or spectroscopy.
3. The body of release and stability data generated during development, in conjunction with appropriate process controls and pack type, are sufficient such that no control test is required.

Consideration needs to be given to the presence of crystalline content in the formulation. Crystalline content can result from the incomplete transformation from crystalline to amorphous during the drug product manufacturing process or partial transformation back to crystalline API during the drug product manufacture. Another root cause for crystalline content can be the transformation of the amorphous drug substance throughout the shelf life of the product.

Where crystalline content is to be measured as part of the control strategy the choice of detection method (scenarios 1 and 2 above) may depend upon the limit of detection of the potential methods and any knowledge of the in vivo impact of different levels of crystalline content.

In addition to the dissolution test, direct measurement techniques of crystalline content may be limited by interfering responses due to the presence of a signal from crystalline excipients in the formulation. Nevertheless, in many instances, this may be the more sensitive test. This was reflected in the 2017 survey in which 43% of respondents said they used dissolution during development to gain product understanding compared to 57% who used solid-state techniques such as XRD, DSC, ssNMR, Raman, or NIR as the primary approach to explore the presence of crystalline content. In addition, when asked how crystallinity is monitored for QC purposes, again 43% of respondents used dissolution, 52% use NIR or Raman, 19% did not use a QC test, and 71% referenced "Other" approaches, predominantly XRD and DSC. For this question, respondents were able to select as many of the options that applied, as the answer may be product-specific; hence, the total sums to greater than 100%. A recent IQ Whitepaper (52) discussed in depth the solid-state techniques, analytical challenges, and limitations of detecting low levels of crystalline drug substance in amorphous solid dispersions (ASDs) and associated drug products.

Irrespective of the nature of the procedure used as a control test, reference batches of the formulation with a range of known crystalline contents are required to determine the detection limit of the procedure. However, the manufacture of these reference materials is not a trivial task since the goal is for the properties of the crystalline material within the polymer matrix (e.g., distribution and particle/domain size) to be representative of those formed on stability or residual from the API or drug product manufacturing processes. For those that use dissolution as the means of monitoring or understanding crystalline content, further information was sought in the questionnaire to understand how the discriminatory nature of the method was determined. Approaches included the use of non-sink conditions, spiking the drug product with crystalline API at a range of concentrations, use of process variants intended to facilitate the incomplete transformation to the amorphous form, and stressing the formulation to induce turnover.

In addition to understanding the discriminatory capability of the QC dissolution method, the method parameters should ideally be set to allow a clinically relevant specification to be defined (53). Examples of real-world case studies from each of the three control strategy options described earlier are presented below.

Case Studies demonstrating control strategies for cystalline content in ASDs

Detection and Control of the Stability of the Physical Form by QC Dissolution

For a tablet product containing ASD, a QC dissolution method was developed and employed not only to measure product dissolution, but also as an assessment of the physical form of the drug product both at release and on stability. A quantitative relationship between the percent crystallinity detected and percent dissolved at the proposed specification time point was established.

Experiments were performed to show the ability of the dissolution method to discriminate crystalline content in tablets. First, the ASD was blended with crystalline drug substances at different ratios. Tablets were then manufactured using the resulting blend. A strong correlation between

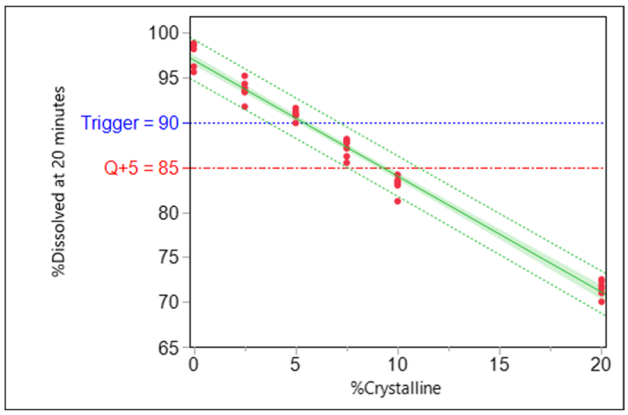

Fig. 5 Dissolution at Q as a function of crystalline content

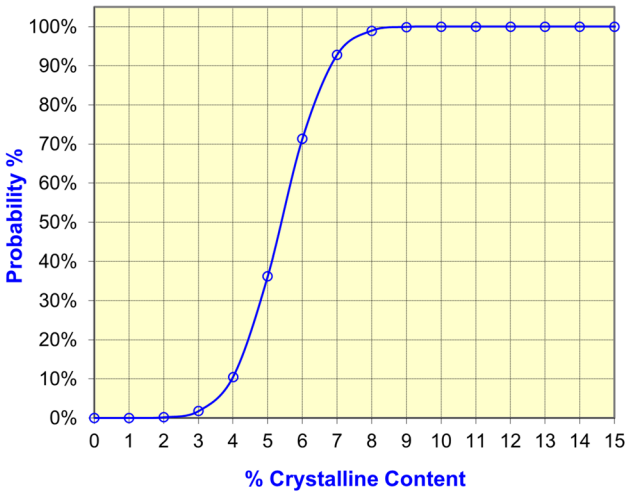

Fig. 6 Estimated probabilities that a lot will trigger XRPD testing for physical form, as a function of percent crystalline content

dissolution and percent crystallinity was observed. A linear model was fitted to the data to predict the percent dissolved at the specification time point as a function of crystalline content. The green shaded area in Fig. 5 is the 95% confidence region for the fitted line, while the green dotted lines are the 95% prediction limits for percent dissolved at the specification time point for future individual tablets. The regression is statistically significant with $p < 0.0001$ and adjusted RSquare > 0.98, confirming that percent crystalline content is predictive of percent dissolved in a statistically significant manner. It is worth noting that the slope does not necessarily equal 1, i.e., the loss in percent dissolved and the growth in percent crystalline may not have a 1:1 correlation. Potential reasons for this include the following: (1) The crystalline form of the material may also have some solubility in the dissolution media selected; (2) crystallization of the ASD may affect other tablet properties that the dissolution method is sensitive to, and the compounded effect could result in more change in dissolution than percent crystalline growth.

The next component of the methodology for control of physical form via dissolution testing is to define a trigger limit such that if the percent dissolved at Q is below this limit, the crystalline material is likely to be present and thus requires direct measurement of the physical form via XRPD testing. This is essentially a two-staged approach, with dissolution as the first stage of control and XRPD as the next stage if it exceeds the pre-defined limit, as a change in dissolution is not only impacted by crystalline growth but it could also be impacted by other material attributes and process parameters. This trigger limit is derived from release and stability data of historical lots that had been confirmed by XRPD to be fully amorphous; this was set at a mean $(n = 6) < 90\%$ dissolved. This two-staged control strategy has been accepted by all regions where the marketing application was submitted.

The statistical interpretation of the trigger limit is as follows: on the assumption that the means of the available release and stability samples are representative of the percent dissolved at 20 min for amorphous lots, 99.9% of future lots manufactured under the same conditions are expected to have a mean percent dissolved at 20 min that is at this limit or above, and this statement is made with 95% confidence. In other words, a future lot whose mean percent dissolved at 20 min is below 90% dissolved would be inconsistent with the historical range of known amorphous samples and would trigger XRPD testing.

Combining the trigger limit, the predictive model from Fig. 5, and the normal variability of fully amorphous samples, the probability that a given batch of tablets will fail the dissolution trigger limit and require XRPD testing, can be calculated as a function of crystalline content. The probabilities are presented in Fig. 6. The curve shows that XRPD testing would be triggered with a high probability when percent crystallinity reaches 7–8%. Thus, this methodology, which includes the trigger limit, is shown to be a sensitive indicator of the presence of crystalline material.

Detection and Control of Physical Form Stability by Direct Measurement of Crystalline Content

Detection of the crystalline content could also be made by direct measurement with solid-state techniques, such as XRD, DSC, ssNMR, Raman, or NIR. These techniques are, in general, more specific and sensitive (52) than the indirect measurement of crystalline content with the QC dissolution method, therefore easier to justify. However, these techniques are in general less robust than QC dissolution and require not only specialized technique/equipment but also technical expertise that may not be readily available in commercial QC laboratories across the globe. Raman and NIR may also require the use of a calibration model, subjecting it to lifecycle and model maintenance considerations. In addition, the

development and validation of such methods could encounter technical challenges. For example, in a tablet product containing ASD, the crystalline form generated from forced crystallization of ASD was in a different polymorph than the neat crystalline material. When using the XRPD technique for quantitation, the stressed crystalline form resulted in a much broader, low S/N and low sensitivity peak in X-ray diffractogram, which made method transfer to commercial QC labs quite challenging, as it required a special algorithm to be employed in peak integration. Based on our experience, the direct measurement approach is the most well-received control strategy from a health authority review perspective.

No Test or Control Is Required in Commercial Product

Both direct and indirect measurements were discussed previously; a third option, and the most desired scenario, is that no test is required. Based on ICH Q6A Decision Tree #4 (51) for the drug product, this can be achieved with scientific justification, extensive forced crystallization data, and knowledge obtained during product development, demonstrating that the product made with ASD is physically stable throughout the shelf life and that no change that could affect the safety or efficacy would occur. Specifically, in the case study presented here, the stability of the amorphous form at relevant storage temperatures was estimated using the following approach: The onset time and the rate of the crystallization were obtained for each stress condition as the intercept and slope of a linear least-squares analysis, as illustrated in Fig. 7. The crystallization onset time and the rate at 25 and 30°C were then extrapolated from the high-temperature data with an Arrhenius analysis (54). Subsequently, the cumulative time to reach the control limit of 5% crystallinity was estimated as the sum of the crystallization onset time and the time to 5% crystalline form.

Monte Carlo simulations were then performed to estimate the variability of the extrapolated estimates of cumulative time to reach 5% crystallinity. For each stress condition, simulated Gaussian random errors were added to the observed values of crystallinity at each time point, and the linear regressions and Arrhenius extrapolations described above were repeated 1,000,000 times for each simulated data set. The resulting cumulative probability to reach 5% crystalline at the long-term storage condition is shown in Fig. 8.

The lower 0.1 and 0.01 percentile probability limits are all magnitudes longer than the product's shelf life. The crystallization modeling from these studies at stressed conditions predicts a negligible probability of growth of crystallinity in the drug product over the proposed shelf life. In addition, real-time stability data collected through development also confirmed the physical form stability of the product. No test or control is required for this product upon commercialization. This control strategy has been submitted to multiple regulatory agencies and has been well received.

Conclusion

This manuscript discusses that classical sink considerations are based on crystalline (thermodynamic, i.e., ≥ 24 h) drug solubility. Thus, they are only partly adequate for QC dissolution testing of supersaturating ASD formulations. Instead, assessing the supersaturation concentration of the amorphous form allows the identification of the kinetic sink factor for such formulations. Since ASD formulations have evolved during the last years, there is no standard practice within the industry on how to assess drug solubility that is

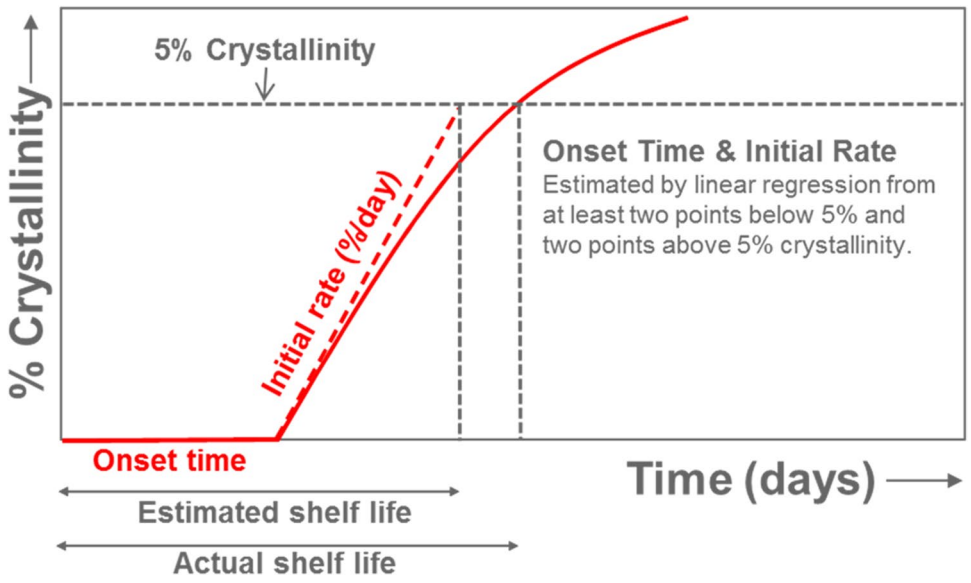

Fig. 7 Forced crystallization data analysis approach

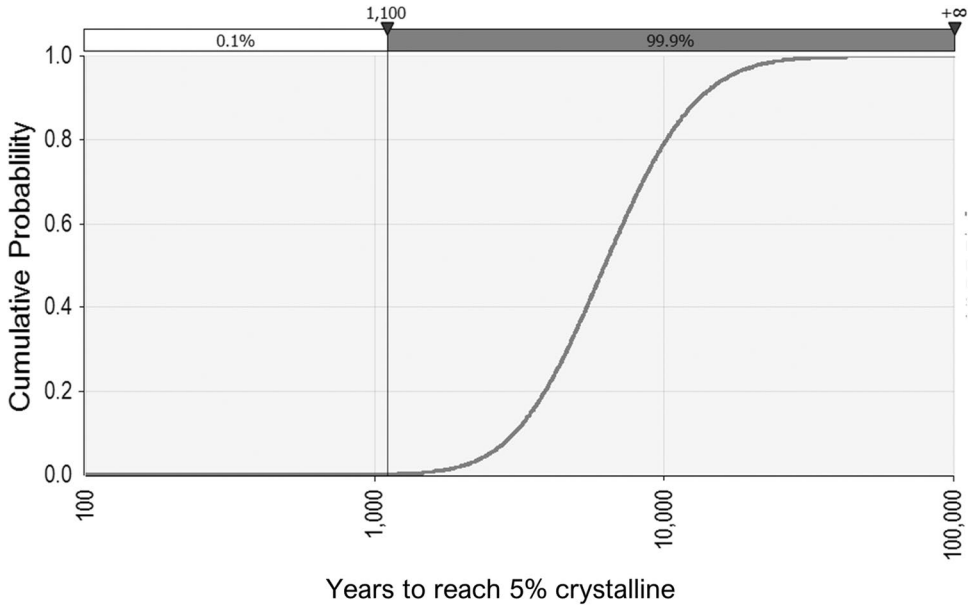

Fig. 8 Likelihood of crystallization at the long-term storage condition

representative for such formulations. Therefore, this manuscript summarizes various methodologies to determine drug concentration for the intended purpose. Given the inherent instability of supersaturated systems, harmonization of in vitro protocols to determine amorphous apparent drug solubility in ASD formulations would substantially increase the reliability and reproducibility of such measurements.

During the survey conducted by IQ members, 50% of the participant companies reported that the proposed dissolution method was not viewed favorably if the method was not capable of detecting crystallization in the ASD formulation. One of the main questions in this manuscript is if in vitro dissolution represents the right tool to detect crystallinity in an amorphous formulation. According to ICH Q6A decision tree 4, there are three main scenarios, i.e., no control required; the control required using solid-state analysis, by in vitro dissolution; or a combination thereof. In the case of in vitro dissolution, the main prerequisite is that the dissolution test can discriminate for the presence of crystalline drug substance. Thus, a certain difference in drug solubility between the amorphous and crystalline forms is essential and must be considered case-by-case additionally taking the strength of a single dosage unit into account. Once a dissolution test has been developed and designed to detect the crystalline drug in an amorphous formulation, it can serve as a valuable tool also during lifecycle management. However, the limitations of utilizing dissolution to detect form changes need to be considered in such case. In case solid-state analysis is used as a control, we suggest providing data on the sensitivity of the solid-state method applied and appropriate justification for why in vitro dissolution is not applied. Finally, in case neither QC dissolution nor solid-state methods are used to control the physical form, data on physical form stability, e.g., via extensive forced crystallization studies, should support this decision.

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Author Contribution All authors contributed to the content, review, and editing of the manuscript.

Declarations

Conflict of Interest The authors declare no competing interests.

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