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2020-08-30
Elsevier B.V.
http://hdl.handle.net/10138/330612
Peltonen, L & Strachan, C J 2020, 'Degrees of order : A comparison of nanocrystal and amorphous solids for poorly soluble drugs', International Journal of Pharmaceutics, vol. 586, 119492. https://doi.org/10.1016/j.ijpharm.2020.119492

Review

Degrees of order: a comparison of nanocrystal and amorphous solids for poorly soluble drugs

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Declarations of interest: None

Abstract

Poor aqueous solubility is currently a prevalent issue in the development of small molecule pharmaceuticals. Several methods are possible for improving the solubility, dissolution rate and bioavailability of Biopharmaceutics Classification System (BCS) class II and class IV drugs. Two solid state approaches, which rely on reductions in order, and can theoretically be applied to all molecules without any specific chemical prerequisites (compared with e.g. ionizable or co-former groups, or sufficient lipophilicity), are the use of the amorphous form and nanocrystals. Research involving these two approaches is relatively extensive and commercial products are now available based on these technologies. Nevertheless, their formulation remains more challenging than with conventional dosage forms. This article describes these two technologies from both theoretical and practical perspectives by briefly discussing the physicochemical backgrounds behind these approaches, as well as the resulting practical implications, both positive and negative. Case studies demonstrating the benefits and challenges of these two techniques are presented.

Keywords: amorphous, crystallinity, nanocrystals, poor solubility, solubility enhancement, supersaturation

1. Introduction

Active pharmaceutical ingredients (APIs) are most commonly formulated as crystalline (morphous) solids in dosage forms, where the API molecules exhibit long-range order in all directions (with various symmetry operators) and form single or polycrystals on the micron scale. Frequently, the same molecules can be organized in different arrangements, and this ability of a solid material to exist in more than one crystal form, is termed polymorphism. Among drug materials, polymorphism is very common and numerous polymorphs may exist for a single molecular species. For example, indomethacin has at least seven different polymorphic forms (Surwase et al., 2013).

In crystals, the regular atomic and molecular pattern, is thermodynamically stabilized through molecular conformations and intermolecular interactions that minimize Gibbs free energy. The relative stabilities of different molecular arrangements (polymorphs) and conformations, are dictated by the potential energy landscape at a given temperature and pressure. While all crystalline polymorphs occupy local minima on the potential energy surface, the polymorph with the lowest free energy is that occupying the global minimum on the potential energy surface. Theoretically, in crystals all the atoms are perfectly positioned in the repeating pattern. However, in reality, the crystals exhibit a variety of crystal defects, such as dislocations and point defects, which interrupt the crystal structure. These crystal defects are sites of both higher energy and lower mechanical strength. As a result, particle breakage, for example in nanomilling, is more likely to take place in these areas. Furthermore, polymorphic transformations, are likely to proceed from these points, should the sample be subjected to conditions favoring transformation to another crystal form.

On the other hand, amorphous (non-crystalline) solids lack long-range order with respect to their atomic level structure. The molecules in amorphous APIs are often described as being randomly arranged. In real material science, materials are very rarely pure crystalline or pure amorphous (completely random) in structure. An example of a 100% pure crystalline material without any crystal defects is single-crystal silicon, which is widely utilized in silicon-based discrete components and integrated circuits in electronic equipment and solar cells. However, in pharmaceutics, pharmaceutical solids are always positioned somewhere on the continuum of order (between randomly arranged molecules and perfectly single crystals). In most amorphous solids there is usually some short-range order (e.g. dimer formation due to directional intermolecular hydrogen bonding), and in crystalline solids there is always some discontinuity in the structure (Figure 1). Complicating the matter still further, the degree of order may not be uniform across the particle or sample. For example, the surfaces of the particles frequently exhibit a different degree of crystallinity to the core of the particle. This poses challenges not only in the classification of materials as crystalline or amorphous, but also in their analysis with different analytical techniques, which are inherently sensitive to different length-range orders and sampling volumes. Thus, the analytical limits of the techniques are increasingly qualified, by, for example, referring to a material as X-ray amorphous, rather than simply amorphous.

Perfectly amorphous and crystalline solids are two extreme cases of solids, but, as mentioned above, real materials are situated somewhere between these two extremes (Figure 1). Nanocrystals can be considered intermediate formulations, whose physical properties are also intermediate, and depend on the crystal size.

The definition of ‘short-range’ vs long-range’ which divides amorphous from crystalline solids is subject to interpretation. Crystalline materials have, for example, been depicted as including order (symmetry operators) over at least 1000 individual molecules (Bellantone, 2014). If one considers an ‘average’ small drug molecule such as indomethacin (with a Mw of 357. 8 $g \cdot mol^{-1}$), the asymmetric unit of the gamma crystal form contains a single molecule, and the unit cell contains two molecules and has axis lengths of approximately 1 nm. One thousand ordered molecules in a single direction would result in a single crystal size of approximately 500 nm. However, pharmaceutical nanocrystals, are commonly defined as having

crystals sizes of down to 100 nm or less, typical sizes in commercialized products being 200-500 nm, and indeed indomethacin nanocrystals with sizes of 300-400 nm has been prepared and characterized as crystalline, using, for example x-ray powder diffraction and differential scanning calorimetry (Liu et al., 2011; Liu et al., 2015). Thus, pharmaceutical solids characterized as amorphous contain much shorter lengths of order, and indeed short range order is commonly described as the predominance of molecular dimers or trimers, involving, for example, directional hydrogen bonding between specific molecular (Strachan et al., 2007). The molecules in such amorphous solids will inevitably occupy a distribution of molecular conformations and intermolecular interactions about the average short-range structure.

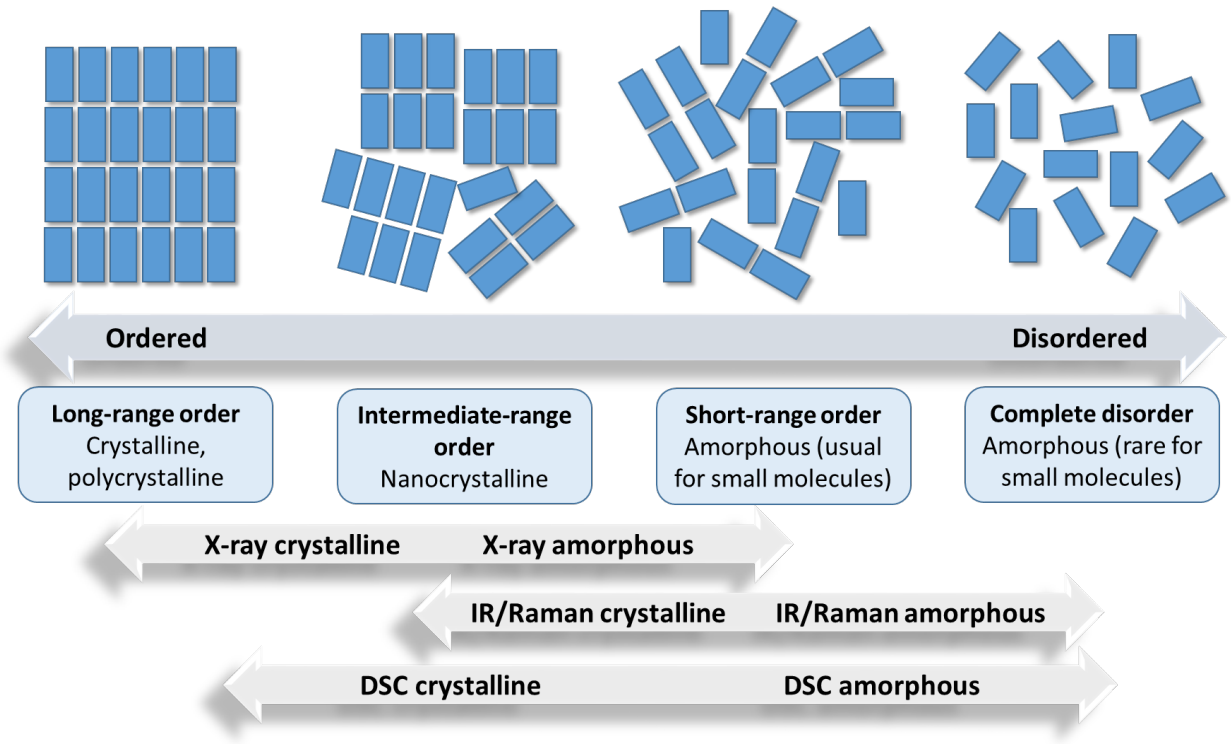

Figure 1. Simplified schematic representing degrees of order in pharmaceutical small molecular solids (not to scale) and their classifications, as well as the concept of different sensitivities of commonly employed analytical techniques. Modified from Bellantone (2014).

Though the chemical composition of different crystalline and amorphous forms of a compound are the same, numerous physical properties, such as solubility, physical stability, density, and thermal and optical properties, differ. Crystals are thermodynamically stabilized by their long-range order, but the disordered amorphous form is a high-energy form and the bonding forces between the molecules are weaker. Of critical importance in pharmaceutics, this induces both higher solubility as well as dissolution rate. In nanosized crystals, more commonly in pharmaceutics referred to as nanocrystals, the solubility is also increased, while the higher surface-to-volume ratio also increases the dissolution rate (Parks et al., 2017). Both the amorphous form and nanocrystals lead to a supersaturated solution, i.e. the apparent solubility is higher than the thermodynamic solubility of the system. The main drawback of both the amorphous form and nanocrystals are their physical instabilities, which is the biggest challenge in their formulation. In amorphous solids, the challenge is to avoid crystallization, while with nanocrystals, uncontrolled aggregation and an increase in particle size needs to be avoided.

When considering the best formulation approach for a poorly soluble API, many alternative solubility enhancing formulation approaches to the amorphous form and nanocrystals exist, including salts, co-crystals, cyclodextrins, surfactants, co-solvents, and different kinds of lipid systems. In these cases, the higher apparent solubility is reached via solubilization. However, these systems have their own limitations. Firstly, they feature specific API molecular property prerequisites (e.g. ionizable or co-former groups, or sufficient lipophilicity). Secondly, if the higher solubility is reached via solubilization, it does not necessarily lead to higher permeation if the drug affinity to the solubilizer is such that it inhibits drug release from the solubilizer system into the physiological environment and subsequent permeation (Dahan et al., 2016). The practical implications of the latter challenge are illustrated with the example of etoposide. When this API was formulated as four different solubility enhancing formulations (cyclodextrin-, surfactant-, and solvent-based formulations, as well as amorphous solid dispersion (ASD)), and the formulation effect on apparent solubility and permeability was investigated, all four formulations increased the apparent solubility. However, only the ASD formulation exhibited a higher apparent permeability (Beig et al., 2015). Accordingly, any enhanced *in vivo* absorption depends upon whether the higher aqueous solubility is reached via solubilization or supersaturation (Kuentz 2019). In addition, when permeation is via active transportation, for example with Pgp, the higher solubility can saturate the efflux transport system and hence increase the permeability (Dahan et al., 2016).While the amorphous form and nanocrystals are often simultaneously considered as potential formulation approaches during the industrial development of a poorly water-soluble API, the number of articles comparing these two techniques for solubility enhancement is very low. Two excellent review articles have been published in which the scientific backgrounds of nanocrystals and amorphous solid dispersions are presented, with the main focus being on formulation and processing, as well as marketed products and patent applications involving these methods (Brough and Williams, 2013; Jermain et al., 2018), and also reviews handling either nanocrystals (Müller et al. 2011, Li et al. 2016, Bhakay et al. 2018, Peltonen and Hirvonen 2018) or amorphous formulations (Grohganz et al., 2013, Laitinen et al., 2014, Edueng et al., 2017) are to be found.

In this context one naturally poses the question, as to whether sufficiently stable amorphous nanosized-particles can be prepared and utilised, to simultaneously harness benefits of both disorder and nano-sized particles. Nanoplexes, in which the nanosized amorphous particles are prepared and stabilised by complexing an ionised drug with a polyelectrolyte indeed show dissolution benefits for certain drugs (Cheow and Hadinoto, 2012, Lim et al., 2017). However, they rely on the drug being ionisable (like salts).

This review focuses more generally on the physicochemical and pharmaceutical manifestations of degree of order of pharmaceutical solids, without any requirement for ionisation, starting from crystalline material, via nanocrystals to the amorphous form. The corresponding characterization methods and associated benefits and challenges are reviewed, different formulation considerations are briefly considered, and finally, the importance of the characteristic physical properties of these different systems for efficient drug delivery is discussed with case studies.

2. Crystalline solids

As described above, crystalline materials exhibit long-range positional and orientational order, and different molecular arrangements result in polymorphs, which are almost ubiquitous for drug materials (Censi and Di Martino, 2015). Polymorphism strongly influences many physical properties, most importantly dissolution and solubility, which in turn, can affect bioavailability (Figure 2).

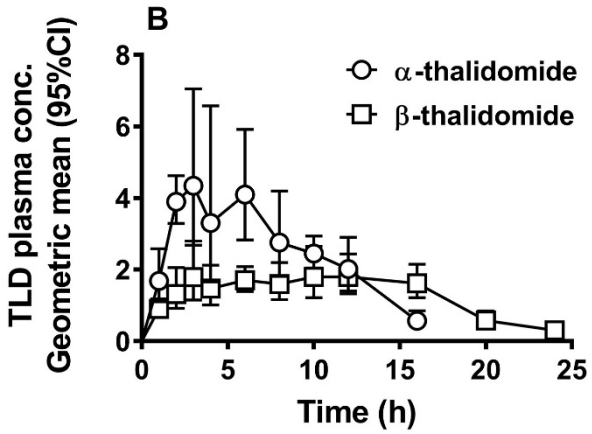

Figure 2. Plasma concentration levels of thalidomide from two different polymorphs as a function of time after oral administration Reprinted from the European Journal of Pharmaceutical Sciences, 136, de Oliveira, G.H.O., do Nascimento, S.B., de Oliveira, F.M., Belo, V.S., de Alencar Danda, L.J., Soares-Sobrinho, J.L., Fialho, S.L., Bedor, D.C.G., de Castro, W.V., 2019. Systematic evaluation of the impact of solid-state polymorphism on the bioavailability of thalidomide, 104937, Copyright (2019) with permission from Elsevier.

Utilization of high throughput crystallization has increased the number of known drug polymorphs, but the searches are not exhaustive, and, based on current technologies, it is difficult to exclude the possibility of later discovering further polymorphs. Surwase et al. (2013) studied the effect of pH and temperature on indomethacin crystallization behavior in aqueous suspensions containing amorphous indomethacin particles, and serendipitously discovered that, in addition to the pH and temperature influencing the crystallization rate, new polymorphs also appeared. When the samples were held at 5 °C, depending on the pH, three new polymorphic forms, ε, ζ, and η, were sequentially observed (Figure 3). Their existence was confirmed by DSC, XRPD, FTIR, and Raman spectroscopy at various sampling times.

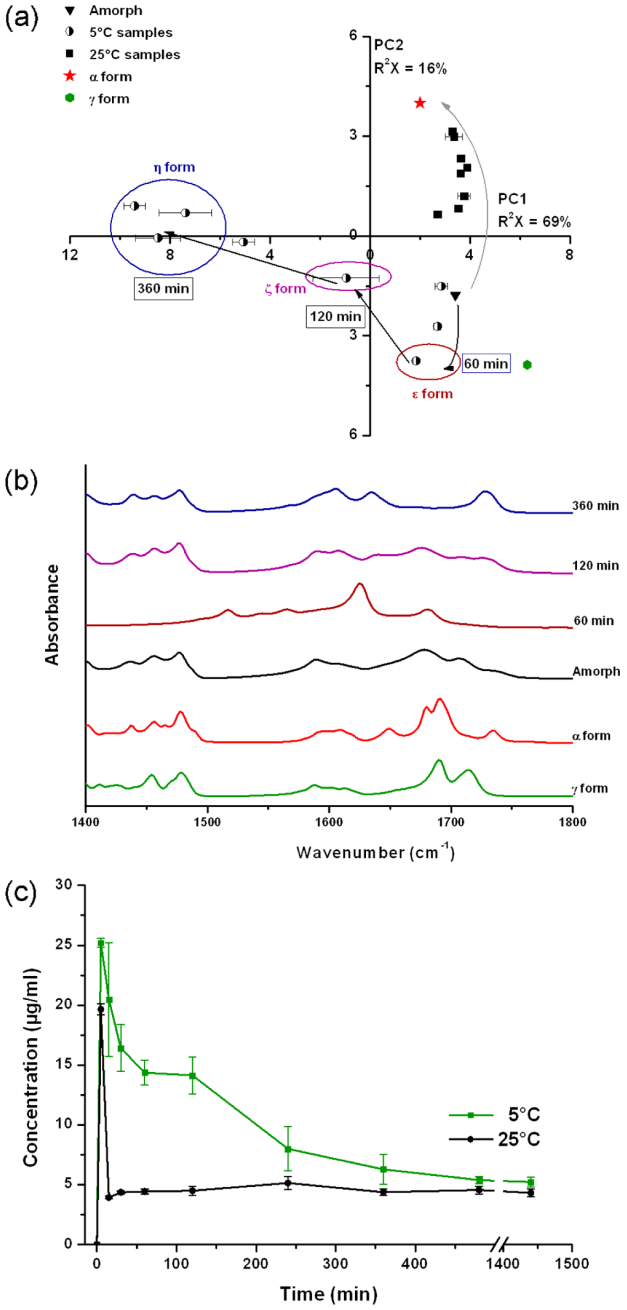

Figure 3. Crystallization path and solution concentration levels of amorphous indomethacin suspensions in pH 1.2 at different temperatures. (a) IR spectra PCA scores plot of samples at different time points, (b) IR spectra of reference samples and from the suspension at 5 °C at certain time points, (c) solution concentration as a function of time. Reprinted with permission from (Surwase, S.A., Boetker, J.P., Saville, D., Boyd, B.J., Gordon, K.C., Peltonen, L., Strachan, C.J., 2013. Indomethacin: new polymorphs of an old drug. Mol. Pharmaceutics 10, 4472-4480). Copyright (2013) American Chemical Society.

In some cases, the solubility of different polymorphs are sufficiently similar that no real differences are seen in bioavailability values. However, the well-known example of ritonavir, demonstrates that the appearance of a previously unknown polymorph can almost totally hinder the drug absorption due to dramatically lowered solubility (Chemburkar et al, 2000; Bauer et al., 2001). In 1998 Norvir, a semi-solid capsule formulation of ritonavir, failed in control tests with crystals appearing in the formulations. Earlier, from the beginning of the discovery phase of the drug's development until the new drug application (NDA) filing, only one crystal form of ritonavir (form I) was known to exist. The crystal structure of ritonavir was thoroughly

analyzed using solid state nuclear magnetic resonance (SS-NMR) spectroscopy, near infrared (NIR) spectroscopy, X-ray powder diffraction (XRPD) and single crystal X-ray techniques (Bauer et al., 2001) and identified as a new form II, which has substantially lower solubility. After discovering form II, its heterogeneous nucleation mechanisms were studied. Polymorphic form II exhibits in molecular level a *cis* conformation, which has more stable packing associated with a strong intermolecular hydrogen-bonding network. However, precipitation, even when form II seeds are present, is energetically unfavorable unless the solution is highly supersaturated. The final outcome of the study was that the high level of supersaturation in the solution together with the probable heterogeneous nucleation by a degradation product led to the crystallization of form II with significantly lower solubility. Later, in high throughput crystallization studies, five crystal forms of ritonavir were observed (Morissette et al., 2003). In addition to forms I and II, one metastable polymorph, one hydrated form and one formamide solvate of ritonavir were characterized. In fact, hydrates and solvates are not considered polymorphs. Structurally they can be defined as co-crystals, where the co-crystal former is either water (hydrate) or some other solvent (solvate).

If the particle size of any crystalline form of a drug is decreased below the micrometer size range, the material properties begin to change. Drug nanocrystals are solid pure drug particles, where the solid drug core is generally covered by a stabilizing polymer or surfactant layer (Merisko-Liversidge et al., 2003; Van Eerdenbrugh et al., 2008; Shegokar and Müller, 2010; Peltonen and Hirvonen, 2018). Nanosized solid particles tend to aggregate very easily, and the polymer or surfactant layer is needed to stabilize the particles from aggregation (Wang et al., 2013). The layer also hinders Ostwald ripening. The physical stability of nanocrystals mostly relates to the maintenance of particle size, but polymorphic changes (Lai et al., 2011) or formation of amorphous material (typically with anti-solvent precipitation or liquid atomization based nanocrystallization techniques) (Matteucci et al., 2007) may take place during the production of drug nanocrystals, which necessitates careful attention from physical stability point of view. The feasibility of nanocrystallization techniques is demonstrated by the fact that the total number of drug applications containing nanocrystals for US Food and Drug Administration (FDA) reached over 80 by the beginning of 2017 (Chen et al., 2017).

Typical stabilizing polymers for drug nanocrystals include different kinds of cellulose derivatives (Tuomela et al., 2014; Ito et al., 2016), polyvinyl pyrrolidone (PVP) (Nakach et al., 2016), poloxamers (Liu et al., 2015), and vitamin E TPGS (Ghosh et al., 2012). Surfactants such as polysorbates (Li et al., 2018) or sodium dodecyl sulphate (SDS) (Afolabi et al., 2014) have also been used, either alone or in a combination with polymers. NMR studies have shown that the nanocrystal particle is formed from a solid drug core, which is surrounded by a semisolid phase of drug and stabilizer (Figure 4) (Kojima et al., 2018). The semisolid phase is in equilibrium with the surrounding solution phase and is responsible for stabilization of the particle size.

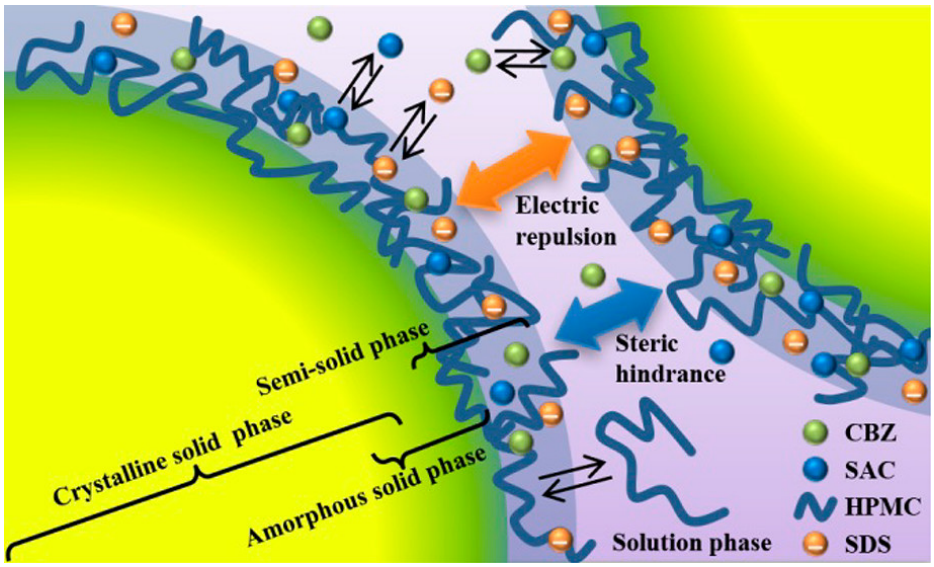

Figure 4. Schematic presentation of the crystalline solid core, amorphous solid phase and semisolid phase on the nanoparticle surface. Semi-solid phase is in equilibrium with the solution phase and it stabilizes the nanoparticle by steric hindrance and/or electrostatic repulsion. (CBZ: carbamazepine drug, SAC: saccharose, HPMC: hydroxypropyl methyl cellulose, SDS: sodium dodecyl sulphate.) Reprinted with permission from (Kojima, T., Karashima, M., Yamamoto, K., Ikeda, Y., 2018. Combination of NMR methods to reveal the interfacial structure of a pharmaceutical nanocrystal and nanococrystal in the suspended state. Mol. Pharmaceutics 15, 3901-3908). Copyright (2018) American Chemical Society.

Besides stabilizing the nanosized drug particles, stabilizers also increase solubility via improved wetting. Stabilizers are hydrophilic or amphiphilic materials, and when attached to hydrophobic solid drug surfaces, the hydrophobic surfaces are become hydrophilic, enhancing wettability and thus solubility, when compared to bulk drug (Yang et al., 2019). For example, when the solubility enhancing effect of different stabilizers on the solubility of bulk indomethacin was studied, it was found that when the solubility of bulk indomethacin in acetate buffer (pH 5.0) was 4.89 µg/ml, the corresponding solubility values in the presence of polysorbate 80 was 10.90 µg/ml, 6.43 µg/ml with poloxamer F68 and 4.80 µg/ml with poloxamer F127 (Sarnes et al. 2013). Both poloxamers as well as polysorbates are widely utilized stabilizers in the production of drug nanocrystals, and this study clearly indicates the solubility effect of the stabilizers. Also, amphiphilic surfactants may increase the solubility via solubilization. In a study by Sironi et al. (2017), different fenofibrate formulations and their dissolution and permeation behavior were studied. It was demonstrated that nanoparticle formulation showed supersaturation after dissolution. Further, micellar solubilization increased the apparent solubility, although this did not lead to an increased permeation rate. On the other hand, undissolved nanoparticles functioned as a drug reservoir, helping to maintain constant drug flow for permeation.

3. Amorphous solids

As mentioned in the introduction, amorphous solids lack long-range orientational and positional atomic or molecular order (and corresponding symmetry operators), but usually exhibit some degree of short-range orientational and/or positional order. Amorphous solids are often defined as a frozen liquid: at the molecular level the structure is liquid like, while at the macroscopic level, they have viscosities and hardnesses typical for solids. Nevertheless, amorphous and crystalline solids differ in numerous physicochemical properties, including mechanical properties and thermal properties, and most importantly, physical (and chemical) stability and solubility. Of note, the wettability of amorphous drugs is also better than the corresponding bulk crystalline form (Yang et al., 2019). The higher chemical potential of amorphous solids with respect to their

(nano)crystalline counterparts, simultaneously results in the higher apparent solubility of amorphous solids, as well as the driving force for amorphous materials to crystallize.

Amorphous forms drugs are seldom sufficiently kinetically stable in pure form in ambient conditions to enable their use in pure form. While some such products do exist (Wyttenback and Kuentz, 2017), the API molecules in these formulations are typically large and exhibit high degrees of molecular conformational flexibility, which serves to inhibit their crystallization. The bulk of poorly soluble amorphous drugs require physical stabilization, and a desire to store medicines at room temperature precludes using low temperatures to reduce molecular mobility and hence increase stability. Thus, excipients are added to increase amorphous form stability. The three main methods to stabilize the amorphous form are i) polymeric amorphous solid dispersions, ii) mesoporous particles, and iii) co-amorphous systems, involving the API and one or more other small molecule components (Laitinen et al., 2014). Of these, polymeric solid dispersions have the longest history and represent the majority of marketed amorphous drug products. Many excellent reviews about amorphous solid dispersions exist (e.g. Laitinen et al., 2014), and thus only a few aspects to consider with amorphous solid dispersions are mentioned here.

Polymeric solid dispersions are generally formulated to be homogeneous single-phase systems (often referred to as glass solutions), in which the drug is molecularly dispersed in a highly water soluble amorphous (or predominantly amorphous) polymer (e.g. poly(vinyl pyrrolidone, cellulose-based derivatives, or amphiphilic polymers). With amorphous solid dispersions produced in combination with water-soluble polymer, drug- and carrier-controlled release is demonstrated (Craig 2002). Incidentally, the same phenomenon can be seen in loose aggregations of drug nanocrystals, where the stabilizer polymer binding particles together rapidly dissolves during the dissolution releasing the individual drug nanoparticles (Craig 2002; Liu et al., 2011; Liu et al., 2015).

Often, the solubility of the drug in the polymer is exceeded, and thus kinetic stabilization of the amorphous solid dispersion is employed. Stabilization mechanisms include several interrelated phenomena: a) drug-polymer interactions (e.g. hydrogen bonding), b) reduced molecular mobility of the drugs, and c) steric hindrance. Sometimes, some degree of phase separation may be present, with resulting drug- and polymer-rich amorphous phases (often referred to as glass suspensions). Such phase separation can both reduce dissolution and predispose the amorphous dispersion to crystallization during storage and/or administration, and thus an understanding of any phase separation and its consequences is required for product optimization. Challenges of amorphous polymeric solid dispersions include limited drug loadings, relatively high hygroscopicity, and uncontrolled phase separation and ultimately crystallization. Development of new methods to characterize and then predict and prevent drug-polymer phase separation, as well as crystallization, during production, storage and dissolution, is a very broad and active area of research. In particular, predictive approaches to probe drug-polymer solid dispersion formation and stability, are gradually helping to bring more efficiency and reliability to stable amorphous solid dispersion development. Experimental and computational approaches, considering thermodynamic and kinetic aspects to varying degrees, include the use of solubility parameters, Flory-Huggins interaction parameter, molecular descriptors, and increasingly, computational molecular modelling. Computational approaches include molecular mechanics and dynamics simulations, as well as more quantum mechanics based approaches such as density functional theory. While all modelling approaches rely on approximations and assumptions to varying degrees and each has its limitations which must be considered, increasing computational power is likely to permit increasingly accurate simulation of amorphous solid dispersion formation and stability in future (Chakravarty et al., 2017, DeBoyace and Wildfong, 2018).

More recently, co-amorphous forms have become a viable alternative to polymeric amorphous solid dispersions (Laitinen et al., 2012; Löbmann et al., 2017). While single-phase polymeric amorphous solid dispersions could equally be considered to be co-amorphous systems, co-amorphous forms generally refer

to single-phase amorphous systems, in which both the API and co-former are small molecules, with typical co-former molecules including amino acids and citric acid (Laitinen et al., 2012). Thus, co-amorphous forms may be considered the amorphous equivalent of co-crystals. It is interesting however, that co-amorphous forms that are easily prepared do not, as a rule, crystallize into the equivalent co-crystals, and if crystallization occurs, it is to crystals of the individual components. The stabilization and solubility enhancing mechanisms can be considered essentially the same as for the polymeric amorphous dispersions. Importantly, the dissolution of co-amorphous forms is generally higher than that of pure amorphous forms, and this has been considered some detail elsewhere (Laitinen et al., 2017). Potential advantages over polymeric solid dispersions include lower hygroscopicity, possible higher drug loadings, as well as simpler, more precisely defined chemical composition. While no known co-amorphous forms have yet reached the market, this is expected to change in the near future.

The third main formulation approach involves mesoporous systems, principally mesoporous silica particles. These systems involve nanoscale pores that are large enough to load drug within them, but are too small to allow crystallization (including nanocrystallization). Mesoporous formulations are also expected to reach the market in the near future, and the systems are well reviewed elsewhere (Laitinen et al., 2012, Maleki et al., 2017, Bremmell and Prestidge, 2019, Jones and Bimbo, 2020).

4. Characterization of order

The toolbox of methods for solid-state characterization is large. However, the structural continuum from crystalline to amorphous solids, and as well as structural and thermal variation between differently prepared and stored amorphous forms, makes solid state characterization of pharmaceuticals far from simple. Compounding this challenge is the fact that different analytical techniques probe different lengths of order and solid-state phenomena, and thus have different sensitivities. When faced with characterizing the crystallinity of a sample, several questions arise. What technique(s) should be used? How should the data be interpreted? How does one define when the sample is amorphous or crystalline? These questions will be considered below, with the aid of case studies.

The most commonly used solid-state analysis methods in pharmaceutics are x-ray diffraction (XRPD) (often considered the gold standard for crystallinity analysis), some form of vibrational spectroscopy (infrared (IR), Raman, near-infrared (NIR) spectroscopy, or, more recently, terahertz), thermal analysis (differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and isothermal microcalorimetry (IMC)), and microscopy (polarizing light microscopy (PLM), scanning electron microscopy (SEM), and, in the case of nanocrystals, transmission electron microscopy (TEM)). Many book chapters (in e.g. (Müllertz et al., 2016)) and articles review one or a subset of the available solid-state analysis techniques in a pharmaceutical context (e.g. spectroscopy (Strachan et al., 2020), and several overviews of all the most commonly used techniques are also available (Chieng et al., 2011; Pindelska et al., 2017; Ma and Williams, 2019). An overview of the more widely available solid-state analysis techniques is presented in **Table 1**, together with some theoretical features and practical issues.

Table 1. Brief overview of some of the most common techniques for solid-state characterization (modified from Novakovic (2020)).

Technique	Benefits	Drawbacks	Depth resolution (depends on setup)
Particular (assembly of molecules) level properties			
Diffraction-based			
XR(P)D	Crystal form identification, differentiation between amorphous and crystalline/nanocrystalline quantification of crystallinity, non-destructive	Preferred orientation effect, lack of sampling flexibility in e.g. processing environment	Approx. 10-500 µm
Thermal			

DSC	Sensitive including to low levels of crystallinity (seeds in amorphous solids), quantification of crystallinity, small sample amount (~ µg), information about molecular mobility and stability of amorphous solids, thermodynamic and kinetic information	Destructive, inflexible sampling, difficult interpretation, slow	None
Microscopy and electron microscopy techniques			
SEM and AFM	Extremely high spatial resolution (~ 1 nm), small penetration depth, detailed particle morphology/surface information	Not directly solid-state specific, coating and vacuum usually required	Approx. 1 nm or less
PLM	Easy, inexpensive, sensitive to low levels of crystallinity	Limited solid-state specificity, not suited to opaque solid dosage forms	Limited in transmission
Molecular level properties			
Spectroscopic (including microscopy) techniques			
Spontaneous Raman	Signal intensity theoretically proportional to concentration, no sample preparation, can be used in aqueous systems and processing environments	Potentially weak signal, slow imaging, can be hindered by fluorescence, not always sufficiently surface specific	Approx. 5-10 µm (microscope)
ATR-IR	Absorbance theoretically proportional to concentration, relatively surface specific (can also be disadvantage), no sample preparation	Clamp pressure can induce crystallization, water interference, inflexible sampling	Approx. 1-2 µm
ssNMR	Rich qualitative and quantitative solid-state information, sensitive to low levels of crystallinity, information on asymmetric unit in crystals	Slow analysis (hours)	None

Different analytical methods have been shown to provide different results when determining the crystallinity of the same samples. One example of such an analytical challenge concerns the preparation of amorphous material by milling (Mah et al., 2014). Mah et al (2014) used dry milling to convert crystalline glibenclamide into the amorphous form. The solid-state form of the material was characterized after different milling times by X-ray powder diffraction (disappearance of diffraction peaks), Raman spectroscopy (spectral changes detected by principal component analysis of the Raman spectra), and differential scanning calorimetry (change in crystallization onset temperature). According to XRPD, the sample was amorphous after 30 min of milling, while the sample reached maximum disorder after 60 min and 180 min according to the Raman spectroscopic and DSC analyzes, respectively. These differences in sensitivity can be attributed to the different phenomena being probed by these techniques. X-ray powder diffraction detects the presence of a lattice structure and once the length scale of unit cell alignment is insufficient (perhaps less than approximately 5-10 unit cells) for lattice diffraction and constructive x-ray interference, the sample will appear amorphous with x-ray diffractogram characterized by an amorphous 'halo', even though shorter-range order remains. Raman (and infrared) spectroscopy, on the other hand, probes intra- (and inter)molecular vibrations, and thus is theoretically more sensitive to shorter range order than XRPD. Several features, or thermal events, in DSC thermograms can be used as indicators of crystallinity (e.g. presence of a glass transition temperature (Tg), change in heat capacity at the Tg, enthalpy of crystallization, as well as onset of crystallization). In this study, the onset of crystallization showed systematic change for a longer period (up to 180 min of milling) than the other phenomena. This may be attributed to the presence and then loss of residual crystallites or nuclei during milling, which still catalyze crystallization during the DSC measurement, even after this level of order is too low to be detected with the x-ray or vibrational spectroscopy methods employed.

When considering the practical value of the different analytical methods, the link to critical quality attributes is crucial. In this case, the storage stability and dissolution behavior of glibenclamide was probed after different milling times. The onset of crystallization with DSC analysis was the phenomenon that was best correlated with both storage stability and dissolution behavior.

Another factor to consider is sampling volume and crystallinity distribution within the sample. For example, some analytical methods (also depending on the sampling setup) are highly surface biased (e.g. FTIR with an attenuated total reflective (ATR) sampling accessory), while others have no surface bias (e.g. DSC) (Table 1).

Crystallization is frequently surface biased (Yu, 2016; Mah et al., 2017; Novakovic et al., 2018), especially below Tg, and in this situation, the more surface biased techniques tend to suggest faster crystallization than reality (if the whole sample is considered). However, surface crystallinity can be more important than overall crystallinity when considering the critical quality attribute of dissolution, since dissolution occurs at particle surfaces (Novakovic et al., 2020; Priemel et al., 2012).

5. Solubility enhancement

When the crystalline material is converted into nanocrystals or the amorphous form, the concentration during dissolution increases above the thermodynamic equilibrium solubility level and a supersaturated solution is formed (Colombo et al., 2017). This supersaturated solution has excess Gibbs free energy, and thus there is a tendency for the solute to separate from the solution by nucleation and crystal growth, i.e. forming a solid phase. Nucleation and crystal growth are affected by the level of supersaturation, and start after the critical supersaturation concentration value is reached. The level of solubility increase is related to the level of chemical potential of the amorphous or nanocrystalline form and by how much it exceeds the corresponding value for the crystal form. The nucleation and crystal growth kinetics and mechanisms depend on concentration: a lower initial concentration favors particle growth instead of nucleation while higher initial concentrations favour nucleation (Haruta and Delmon, 1986). *In vivo,* this is reflected in competing actions between permeation/absorption and precipitation. Precipitation can also be affected by the presence of polymers or other excipients (e.g. small molecule co-amorphous coformers) into the system (Xu and Dai, 2013; Sarode et al., 2014). Formation of a drug-polymer (or other excipient) mixture decreases the chemical potential of the amorphous drug. These precipitation inhibitors can inhibit or delay the drug precipitation, allowing better absorption.

Fast dissolution followed by supersaturated solution and precipitation are described by spring and parachute theory (Figure 5). The spring occurs when a supersaturated and thermodynamically unstable solution of a drug is produced from a higher energy solid-state form or an extremely fast dissolving form of the material (amorphous form, nanosized particles). If the drug precipitates, the supersaturation (spring) is lost. However, by adding precipitation inhibitors, higher concentration levels can be maintained (parachute), and maintaining the supersaturation for a sufficient period of time can lead to a higher absorption *in vivo* and increased bioavailability. In Equation 1, the degree of supersaturation, *S*, describes the driving force for precipitation

$$S=C_B/C_S\ , \qquad (1)$$

where C_B is the total solute concentration and C_S is the equilibrium concentration of the material. The rule is that the higher the degree of supersaturation, the faster the precipitation.

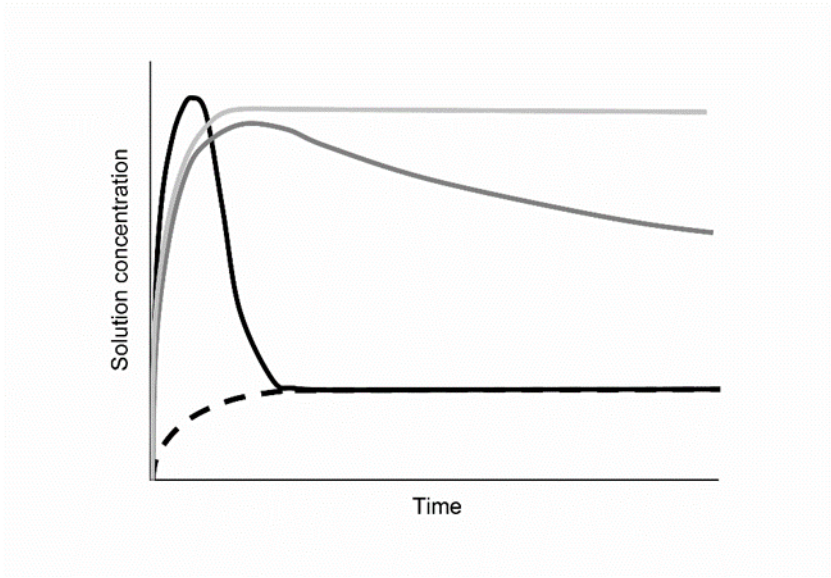

Figure 5. Theoretical presentation of solution concentration as a function of time for i) crystalline drug (black, dashed), ii) pure amorphous drug (black), and solid dispersion with two different polymers: iii) polymer A (medium grey) and iiii) polymer B (light grey). From the figure, it can be seen that all three amorphous formulations have a 'spring effect'. The difference between the two polymers is that the polymer A exerts a parachute effect while polymer B completely inhibits recrystallization and maintains the original degree of supersaturation. Reproduced from (Laitinen et al., 2014) with permission from Springer.

Typical precipitation inhibitors are polymers, mostly studied being cellulose derivatives (MC, HPC, HPMC, HPMCAS) and vinyl polymers (PVA, PVP, PAA (polyacrylic acid, polyvinylpyrrolidone vinyl acetate (PVPVA), but also surfactants, cyclodextrins and potentially co-amorphous form formers (Figure 6).

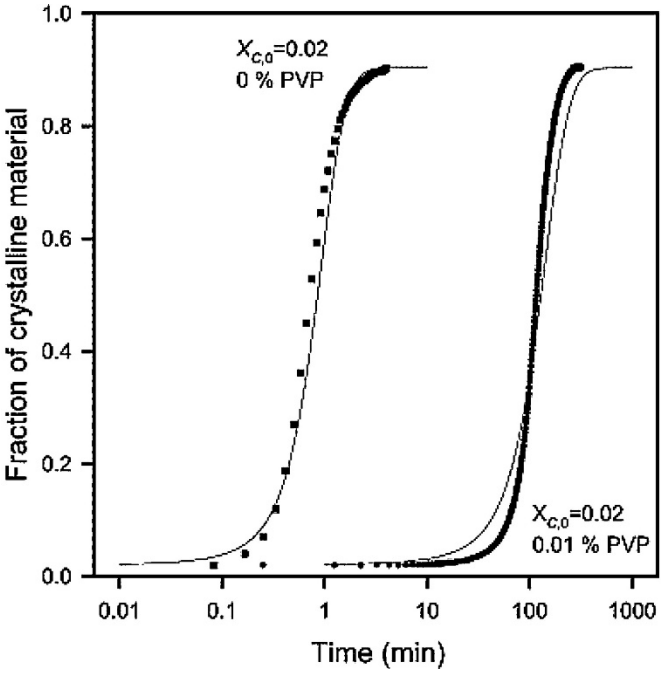

Figure 6. Effect of PVP on crystal growth of bicalutamide in supersaturated solutions. After addition of crystalline nanoparticles to supersaturated solutions, presence of PVP (right curve) delays the crystal growth. Reprinted from International Journal of Pharmaceutics, 453, Xu, S., Dai, W.-G., Drug precipitation inhibitors in supersaturable formulations 36-43, Copyright (2013), with permission from Elsevier

Mechanistically, precipitation inhibition can be divided into two different groups: thermodynamic or kinetic inhibition (Xu and Dai, 2013). Thermodynamic inhibition is based on higher solubility, which lowers the degree of supersaturation. Surfactants and cyclodextrins behave in this way. Kinetic inhibition is reached via inhibition or retardation of precipitation, for example via interfering with crystal nucleation and/or crystal growth. In general, polymers inhibit precipitation kinetically, due to interactions with the drug material or other changes in the solution environment. For example, polymers can adsorb onto the drug particle surfaces. Interactions can be hydrogen bonding based, ionic, or hydrophilic/hydrophobic interactions. Rigidity/flexibility of the polymer chain, as well as molecular weight and viscosity may have an effect, the interplay of which is not yet fully elucidated.

There is growing evidence that the molecular interactions between the drug and the polymer have the most significant impact on the saturation solubility enhancement (Al-Obaidi et al., 2013; Fu et al., 2018; Li and Taylor, 2018). Baghel et al. (2016) studied various amorphous solid dispersion systems with drug-polymer combinations, and found that those that were capable of forming interspecies hydrogen bonds in the solution state were most effective in prevention of drug crystallization. Similarly, with different grades of hydroxypropyl methylcellulose, the presence of hydroxypropyl groups that are capable of hydrogen bonding and strong drug-polymer interactions was the most important property for maintaining supersaturation (Hong et al., 2018). In the same way, the saturation solubility of amorphous griseofulvin was improved with the addition of hydroxypropyl methylcellulose acetate succinate (HPMCAS) due to hydrogen bonding, but increase in the saturation solubility with polyvinyl pyrrolidone (PVP) was much lower (Al-Obaidi et al., 2013). The precipitation of amorphous carbamazepine has been inhibited by interactions between the acetate and succinate groups of hypromellose acetate succinate (HPMC-AS) and carbamazepine (Ishizuka et al., 2019). Acetate and succinate groups disturbed intermolecular carbamazepine interactions in amorphous carbamazepine by forming polymer carbamazepine interactions with C=O and NH_2 groups in carbamazepine. Precipitation inhibition may also be based on hydrophilic/hydrophobic interactions, as was the case when the higher polymer hydrophobicity led to increased amorphous stability of ethenzamide (Frank and Matzger, 2019).

It has also been shown that the theoretically calculated free enthalpy of mixing correlated positively with maintaining the supersaturated state (Price et al., 2019). However, formulation factors and process conditions, including mixing homogeneity of the drug and polymer, affect interaction formation and hence also the dissolution behavior of amorphous solid dispersions (Chen et al., 2016). Also the production method (spray-drying vs. milling or mixing vs. predissolving) affected the degree of supersaturation with the same polymer-drug mixtures, probably via structural effects of the production method (Surwase et al., 2015). Changes in pH conditions can also affect supersaturation maintenance (Xie et al., 2017).

Though with poorly soluble drug materials the rate limiting step for maximum absorption is the dissolution rate, it is good to be aware of that if the solubility is increased dramatically via supersaturated formulations, the rate limiting step may shift from solubility to absorption rate. Accordingly, stabilization of drug solubility (supersaturated state) for a longer time period is very important.

6. Performance in formulations: a comparison of amorphous drug and nanocrystals

As already mentioned, in pharmaceutical research the increasing interest in using the amorphous form and nanocrystals is based on their ability to improve solubility properties, and both these systems are based on higher solubility via supersaturation (Kawakami, 2012; Taylor and Zhang, 2016). Both techniques have been used for solubility improvement (Table 2) and some studies have been published in which the two approaches have been compared. In a study by Zhang et al. (2013), an amorphous solid dispersion formulation of itraconazole outperformed nanocrystal system (Zhang et al., 2013). However, another study

suggested that nanocrystals can outperform amorphous solid dispersions with respect to fast dissolution of low-dose drugs (Li et al., 2017). Both these studies are described in more detail below.

Table 2. Some selected examples of nanocrystalline and amorphous based commercial drug products (modified from Bobo et al. 2016, Chen et al. 2017, Wyttenbach and Kuentz 2017, Malamatari et al. 2018, Peltonen and Hirvonen 2018).

Product	**Active pharmaceutical ingredient**	**Matrix former**	**Production technology**	**Formulation**
Nanocrystalline				
Gris-PEG®	Griseofulvin	-	Coprecipitation	Oral tablet
Naprelan®	Naproxen sodium	-	Media milling	Oral tablet
Verelan®PM	Verapamil	-	Media milling	Oral capsule
Emend®	Aprepitant	-	Media milling	Oral capsule and oral suspension
,Azopt®	Brinzolamide	-	Media milling	Ocular suspension
Triglide®	Fenofibrate	-	Jet-stream homogenization	Oral tablet
Invega Sustenna®	Paliperidone palmitate	-	High-pressure homogenization	Injection (im)
Ryanodex®	Dantrolene sodium	-	Not available	injection (iv)
Amorphous (pure drug)				
Ceftin®	Cefuroxime axetil	-	-	Oral tablet
Viracept®	Nelfinavir mesylate	-	-	Oral tablet
Accupril®	Quinapril hydrochloride	-	-	Oral tablet
Crestor®	Rosuvastatin calcium	-	-	Oral tablet
Accolate®	Zafirlukast	-	-	Oral tablet
Amorphous (solid dispersion)				
Intelence®	Etravirine	HPMC	Spray drying	Oral tablet
Certican®/Zortress®	Everolimus	HPMC	Spray drying	Oral tablet
Fenoglide®	Fenofibrate	PEG	Spray melt	Oral tablet
Gris-PEG®	Griseofulvin	PEG	Melt extrusion	Oral tablet
Sporanox®/ Onmel®	Itraconazole	HPMC/ PVP VA 64	Spray layering (bead coating)/ Melt extrusion	Oral tablet / Oral tablet
Kalydeco®	Ivacaftor	HPMCAS	Spray drying	Oral tablet
Kaletra®	Lopinavir and Ritonavir	PVP VA 64	Melt extrusion	Oral tablet
Cesamet®	Nabilone	PVP	Melt extrusion	Oral capsule
Afeditab® CR	Nifedipine	Poloxamer or PVP	Melt/absorb on carrier	Oral tablet
Nivadil®	Nilvadipine	HPMC	n.a.[a]	Oral tablet
Nimotop®	Nimodipine	PEG	Spray drying/fluid bed	Oral tablet
Noxafil®	Posaconazole	HPMCAS	Melt extrusion	Oral tablet
Norvir®	Ritonavir	PVP VA 64	Melt extrusion	Oral tablet
Prograf®/ LCP-Tacro®	Tacrolimus	HPMC/ HPMC	Spray drying/fluid bed/ Melt granulation	Oral capsule/ Oral tablet
Incivek®/Incivo®	Telaprevir	HPMCAS	Spray drying	Oral tablet

Rezulin®[b]	Troglitazone	PVP	Melt extrusion	Oral tablet
Zelboraf®	Vemurafenib	HPMCAS	Coprecipitation	Oral tablet
Isoptin® SR-E 240	Verapamil hydrochloride	HPC/HPMC	Melt extrusion	Oral tablet

Of course, even higher benefits can be obtained, if the amorphous drug is formulated as nanosized particles, where the high supersaturation solubility based on the amorphous form and fast dissolution due to the large specific surface area of the nanoparticles are combined (Cheow et al., 2014; Bi et al., 2015). While these comparisons clearly demonstrate that both the amorphous form and nanosizing can demonstrate substantially enhanced dissolution, producing the amorphous form or nanocrystals is only the first step, and the role of the final formulation and careful formulation planning is required for successful and optimized *in vivo* performance of these systems.

Sarnes et al. (2014) produced solid oral itraconazole nanocrystal formulations. Itraconazole nanocrystals were produced by wet milling and then either freeze-dried or granulated and, finally, incorporated into tablet or capsule formulations. The *in vitro* drug release for nanocrystalline formulations was immediate and much faster than the commercial product (Sporanox® capsules), which is composed of pellets covered by the drug in an ASD. But, *in vivo*, the relative bioavailability of the nanocrystal formulation was only 27.6 to 39.9% of that of Sporanox®. The key for successful *in vitro/in vivo* correlation with nanosystems requires an understanding of solubilization, precipitation inhibition as well as stabilization of supersaturation, in which immediate release itraconazole nanocrystal formulations failed in this study. The fast dissolution of the itraconazole from the nanocrystals was immediately followed by precipitation due to the fast transit time of dissolved itraconazole to the small intestine, where the solubility of itraconazole is approximately 250-fold lower than in the stomach. However, when itraconazole nanocrystals were bound to nanofibrillar cellulose fibers with cellulose binding domains, the relative bioavailability of nanocrystalline formulations increased to between 119 to 128% of the commercial product (Sporanox® granules) (Valo et al., 2011). In this case, the formulation containing nanocrystals bound nanofibrillar cellulose matrices retained the nanocrystals for longer in the optimal dissolution and absorption region of the gastrointestinal tract.

In a comparison of amorphous itraconazole formulations, Yin et al. (2015) formulated itraconazole as an amorphous form in solid dispersion by supercritical fluid technology. In this study, the relative bioavailability of the itraconazole amorphous solid dispersion was 120% when compared to the commercial Sporanox® granules. Together, these above described studies reveal that, with itraconazole, formulations containing either nanocrystals or the amorphous form were able to reach similar levels of bioavailability . The most important take-home message here is that the production of nanocrystals or amorphous form is just a first step. The successful final performance of the product *in vivo* is highly dependent of the final formulation.

Expanding the nanocrystal/amorphous formulation comparison to other drugs, Li et al. (2017) used hydroxypropyl cellulose and Soluplus® in stabilizing wet milled griseofulvin nanosuspensions. After milling, the nanosuspensions were extruded and dry-milled. A wet-milled nanosuspension was also spray-dried. Two kinds of drug formulations were prepared depending on the drug-polymer solubility properties: extrudates with i) nano/micro-crystalline drug particles in a hydroxypropyl cellulose matrix, and ii) an amorphous solid dispersion, with the amorphous drug molecularly dispersed within the Soluplus® matrix. *In vitro*, the nanosystems dissolved faster than the amorphous solid dispersions. In another study (Zhang et al., 2013), an itraconazole-Soluplus® solid dispersion extrudate exhibited faster dissolution and increased oral absorption when compared with an itraconazole nanocrystals.

Mah et al. (2014) studied amorphous glibenclamide produced by milling, as described above in **Section 4**. The amorphous form was confirmed with XRPD, Raman spectroscopy and DSC to have reached maximum

disorder after 180 minutes (Figure 7). When these amorphous samples were subjected to intrinsic dissolution testing using a flow through set-up, after 10 minutes of dissolution the dissolved drug concentration was approximately 2 µg/ml, and after 20 minutes it was approximately 4 µg/ml. Later, glibenclamide was also formulated as drug nanocrystals with poloxamer 188 or HPMC as a stabilizer (unpublished data). The smallest particles (mean particle size approximately 265 nm) were reached with poloxamer 188 as the stabilizer. In the same intrinsic dissolution test equipment and environment, as in the study by Mah et al. (2014) with amorphous glibenclamide, the solution concentration was 2.1 µg/ml after 10 minutes of dissolution testing, and 3.4 µg/ml after 20 minutes. Intrinsic dissolution testing involves the measurement of dissolution from a flat surface and defined surface area, which eliminates the effect of surface area effect (due to the different particle sizes) on dissolution. However, these two studies (Mah et al., 2014; unpublished data) showed corresponding intrinsic dissolution value levels for both amorphous and nanocrystalline glibenclamide.

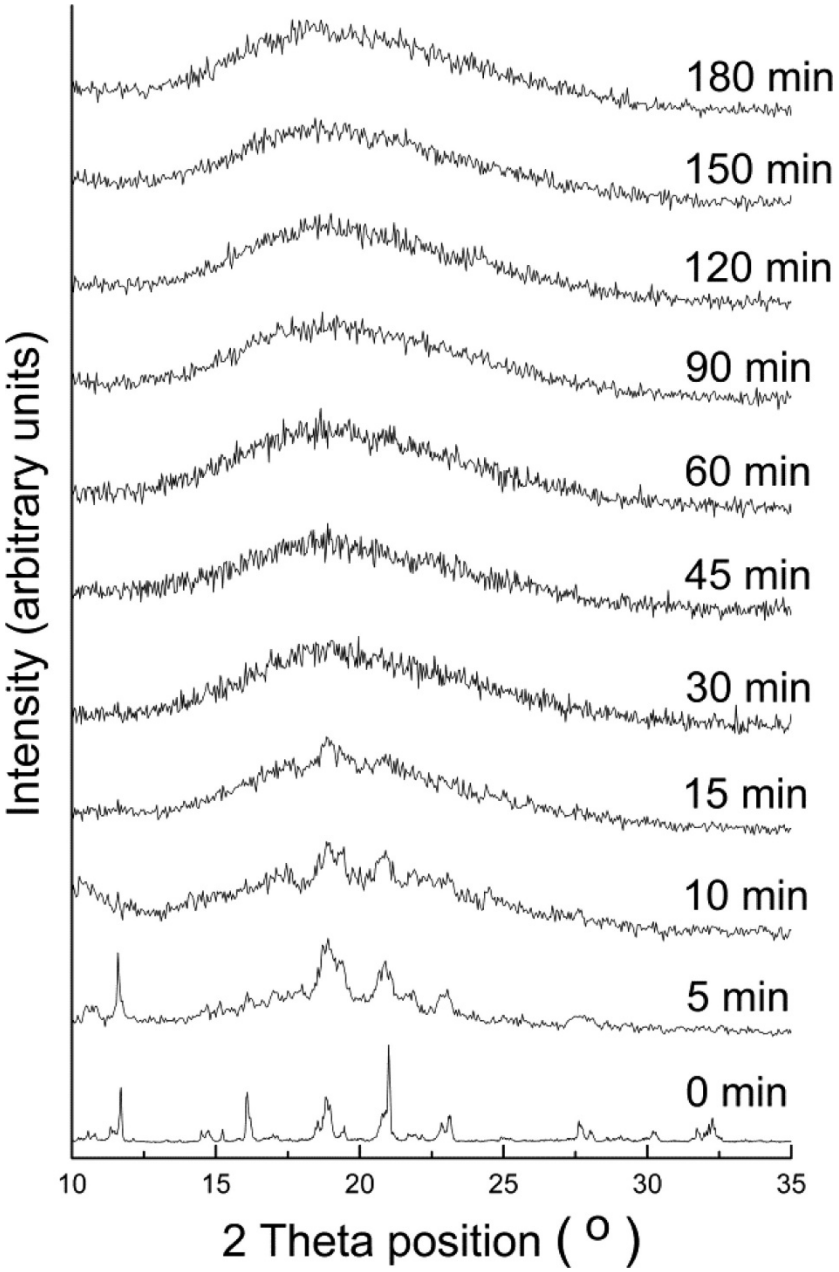

Figure 7. XRPD patterns of glibenclamide samples after different milling times. After 30 minutes milling, glibenclamide is x-ray amorphous, but the sample continued to become more disordered until 60 min according to Raman spectroscopy (as determined by spectral changes detected with the aid of principal

components analysis (PCA)), and 180 min according to DSC (as determined by the increase in crystallization onset temperature). Reprinted with permission from (Mah, P.T., Laaksonen, T., Rades, T., Aaltonen, J., Peltonen, L., Strachan, C.J., 2014. Unravelling the relationship between degree of disorder and the dissolution behavior of milled glibenclamide. Mol. Pharmaceutics 11, 234-242). Copyright (2014) American Chemical Society.

Finally, when the performance of amorphous nanostructured aggregates and wet-milled drug nanocrystals was compared for pulmonary drug delivery, *in vitro* dissolution rates were similar but the amorphous system reached a 4.7-fold higher degree of supersaturation than the nanocrystals (Yang et al., 2010). After inhalation, the *in vivo* lung depositions of both the systems were equivalent. However, the $AUC_{0\text{-}24}$ value of the amorphous system was approximately 3.8-fold higher than that of the nanocrystalline formulation, due to the higher degree of supersaturation and consequent permeation. Of note, the study reported particle sizes (d50 values) of 570 nm for the drug nanocrystals and 250 nm for the amorphous nanosystems. Accordingly, also the smaller particle size of amorphous systems further served to increase the level of supersaturation and subsequent permeation.

7. Conclusions

Most new small molecule pharmaceuticals suffer from poor solubility, and formation of the amorphous form and nanocrystallization are two increasingly popular methods to overcome this challenge. Over the two last decades, research with these two approaches has blossomed and an increasingly large portfolio of commercial products based on these techniques is available on the market. An advantage of these technologies over other solubility or dissolution enhancing methods, is the lack of preconditions with respect to molecular properties. The improvement in solubility with both of these techniques is based on formation of the supersaturated state. However, there are differences between these two techniques in terms of production, characterization, physical stability and formulation approaches. The main advantages of drug nanocrystals are their simplicity and fast production, but the challenge is to avoid aggregation of nanosized particles. With the amorphous form, it is possible to reach very high levels of supersaturation, but the challenge is in stabilization of the amorphous form from crystallization. With both the systems, the maintenance of supersaturation after dissolution requires extra care. The success of both these techniques has already been shown with the increasing number of amorphous or nanocrystalline products entering the market. The number of new drug applications based on these two approaches is expected to continue to increase.

Funding: This review did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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