Drug nanocrystals - versatile option for formulation of poorly soluble materials

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Abstract

Poor solubility of drug compounds is a great issue in drug industry today and decreasing particle size is one efficient and simple way to overcome this challenge. Drug nanocrystals are solid nanosized drug particles, which are covered by a stabilizer layer. In nanoscale many physical properties, like compound solubility, are different from the solubility of bulk material, and due to this drug nanocrystals can reach supersaturation as compared to thermodynamic solubility. The most important effect of the smaller particle size is that dissolution rate is highly enhanced mainly due to the increased surface area. In this review the most important properties of nanocrystalline drug compounds are presented, with multiple examples of the development and characterization of nanocrystalline drug formulations.

Keywords: drug nanocrystals, poor solubility, formulation

Introduction

Today more and more new APIs are poorly soluble, and poor solubility is an important issue in drug discovery and development. Efficient screening methods find increasing numbers of poorly soluble drug candidates for company pipelines and it has been approximated lately that 90% of the new chemical entities are poorly soluble. In Biopharmaceutics Classification System (BCS), these compounds belong to BCS class II (70%) or class IV (20%) (Loftsson and Brewster, 2010; Müller and Keck, 2012).

There are different ways to improve the solubility of compounds. In molecular level prodrugs (Huttunen et al., 2011), salt formation (Serajuddin, 2007), co-solvent systems (Seedher and Kanojia, 2009) or cyclodextrins (Bilensoy and Hincal, 2009) can be beneficial. In particulate level utilization of metastable polymorphs (Blagden et al., 2007), co-crystals (Thakuria et al., 2013), amorphous systems (Babu and Nangia, 2011) or particle size reduction (Tuomela et al., 2015) have been studied intensively. A third approach for solubility/dissolution enhancement are colloidal systems, like SEDDS/SMEDDS/SNEDDS (Thi et al., 2009), (micro/nano)emulsions (Shakeel and Faisal, 2010) or different kinds of other lipid based systems like lipid solutions (Porter et al., 2007). Based on the physicochemical properties of the drug molecule, the most suitable way to improve the solubility should be selected (Shakeel and Faisal, 2010; Chen et al., 2011; Brough and Williams III, 2013).

Drug nanocrystals are solid nanosized drug particles, which are covered by a stabilizer layer, and they are mostly utilized for increasing the solubility properties of poorly soluble drugs (Lu et al., 2016). In BCS system, class II drugs are poorly soluble but well permeable, and those are the most prominent candidates for drug nanocrystals (Liu et al., 2011; Borchard, 2015), but in some cases also BCS class IV drugs, poorly soluble and poorly permeable, may benefit from decreased particle size (Kesisoglou et al., 2007; Gao et al. 2012). For example, a higher concentration gradient between the intestine and lumen (reached with higher and faster drug solubility) may lead also to improved drug permeation.

With a closer focus on drug developability criteria, BCS class II drugs can still be classified into two subclasses according to the developability classification system (DCS): dissolution rate limited class IIa, the so called brick-dust molecules, and solubility limited class IIb, also known as the grease ball molecules (Butler and Dressman, 2010). Brick-dust molecules are poorly soluble not only in aqueous environment but also in lipids and organic solvents, while grease ball molecules are normally soluble in at least some lipids (Bergström et al., 2016). Brick dust molecules are, therefore, the best candidates for nanosizing (Borchard, 2015). For grease ball molecules the first choice is lipid formulations, but they have also been formulated successfully to nanocrystals (Rydberg et al., 2016).

Production of nanocrystals is just one way to modify the intrinsic properties of the raw material: when particle size is decreased to nanosized area, the intrinsic properties like solubility are altered as compared to bulk sized drug powders. The overall benefits connected to small particle size can be divided into three main categories: i) fast dissolution, ii) increased solubility, and iii) better adhesion to membranes. However, absolutely most important effect reached with drug nanocrystals is faster dissolution rate based on the large surface area per mass solid. But, the role of stabilizer and its careful selection should not be neglected. The main role of stabilizer is to stabilize inherently unstable drug nanoparticles against aggregation and/or Ostwald ripening after the production and during the storage of nanocrystalline formulations. However, many stabilizers utilized can for example help in maintaining the supersaturated state *in vivo* reached after fast dissolution of nanocrystals or they may perform as permeation enhancers (Gao et al., 2012; Gao et al., 2014; Chen and Li, 2015; Ueda et al., 2015).

There are excellent earlier reviews related to drug nanocrystals (Müller et al., 2001; Keck and Müller, 2006; Junghanns and Müller, 2008; Müller et al., 2011a; Müller and Keck, 2012; Möschwitzer, 2013; Brochard, 2016; Li et al., 2016). However, often the role of higher saturation solubility and utilization of supersaturation reached with nanocrystalline formulations are poorly described. This review exposes various aspects of drug nanocrystals from all the aspects of basic physicochemical principles to final bioavailability in an integrated fashion. The fast dissolution and increased solubility reached with drug nanocrystals are discussed in detail starting from the physicochemical principles behind drug nanocrystals and ending on formulation examples visioning the broader scope.

1 Characteristics of drug nanocrystals

Drug nanocrystals are solid drug particles surrounded by a layer of stabilizer(s), and sometimes the drug nanocrystals have also been named as solid micelles. Small particle size of hundreds of nanometers makes the nanocrystals unstable and stabilizer(s) are needed to prevent the aggregation of individual nanosized particles. Typical stabilizers are different kind of polymers, like cellulose derivatives, PVP, poloxamers, vitamin E TPGS (Guo et al., 2013; Tuomela et al., 2014; Rahim et al., 2017) or amphiphilic surfactants such as polysorbates, SDS (Liu et al., 2011; Rahim et al., 2017), and these can also improve the solubility via better wetting and solubilizing effects.

1.1 Increased dissolution rate

Increased dissolution rate as compared to bulk drug is the most important effect reached with drug nanocrystals, and it is based mainly on large interfacial area due to the decreased particle size. Taken as an example spherical particles, the surface area versus volume, A/V = 3/r. This means that if the particle size is

reduced from 50 µm (typical particle size for bulk drug) to 500 nm (drug nanocrystals), increase in dissolution rate is 100 fold according to Noyes-Whitney equation (Equation 1):

$$\frac{dC}{dt} = \frac{DS}{Vh}(C_S - C) \quad , \qquad (1)$$

where *dC/dt* is dissolution rate (concentration change as a function of time), *D* is diffusion coefficient, *S* is surface area, *V* is dissolution volume, *h* is diffusion layer thickness, C_s is saturation concentration and *C* is the concentration at time *t*. Accordingly, particle size is an important factor for determination of dissolution rate. However, when the dissolution tests are performed under sink conditions, the differences are typically not seen between different nanocrystal size fractions, and more discriminating dissolution test protocols are needed (Liu et al., 2013).

1.2 Higher saturation solubility

In Noyes-Whitney equation diffusion layer thickness and saturation concentration are also affected by the nanosized particles. For particle size below approximately 50 µm the diffusion layer is starting to get thinner (Sheng et al., 2007), which enhances the dissolution. The increase in saturation concentration is stated in the Ostwald-Freundlich theory (Equation 2), which was first developed for liquid droplets in gas phase, but later it has been shown to be correct also for solid particles in liquid below particle sizes approximately 1 µm:

$$S_{NP} = S_0\left(\frac{2V_m\gamma}{RTr}\right), \qquad (2)$$

where S_{NP} is the solubility of nanoparticles with a radius *r*, S_0 is the solubility of bulk material, V_m is the molar volume, *γ* is the interfacial tension, *R* is the gas constant and *T* is the temperature. The effect of particle size on saturation concentration starts to be seen with particle sizes below 1 µm, but when the particle size is decreased the effect is more pronounced; below 100 nm the increase is even exponential.

Higher dissolution rate and increased saturation concentration leads to supersaturated state, which has been shown to enhance drug permeation (Brouwers et al., 2007 and 2009; Mellaerts et al., 2008). The challenge *in vivo* is the maintenance of supersaturation until the permeation takes place and hindrance of uncontrolled precipitation/crystallization.

1.3 Supersaturated state

Similar to amorphous formulation, due to the higher apparent solubility of drug nanocrystals as compared to thermodynamic solubility, drug nanocrystals produce supersaturated state, which is thermodynamically

unstable (Mah et al., 2015). For example, when drug nanocrystals were compressed to a flat surface, the concentration levels of the dissolved drug next to the sample surface with nanocrystals sized 580 nm was over five-fold higher than concentration levels reached with bulk indomethacin (Figure 1; Sarnes et al., 2013). In another study, aqueous solubility values of nimodipine nanocrystals were determined by a traditional shake flask test (Fu et al., 2013). When the aqueous solubility of crude drug after 72 hours testing was 1.879 μg/ml, for nanocrystals with average particle size of 830 nm, 500 nm and 160 nm, corresponding solubility values were 22.526 μg/ml, 30.093 μg/ml and 51.269 μg/ml, respectively, indicating very high level of supersaturation and also considerably long time period for the system to remain in supersaturated state. Sun et al. (2012) tested kinetic solubility values of nanocrystalline coenzyme Q_{10} with particle size fractions from 80 nm to 700 nm, and the kinetic solubility increased as the particle size decreased. Ueda et al. (2015) analyzed the maintenance of supersaturated state with amorphous and nanocrystalline carbamazepine by real-time NMR spectroscopy by monitoring the amount of dissolved carbamazepine. With carbamazepine nanocrystals concentration values were nearly constant for 50 h time, while with amorphous carbamazepine the initial concentration was higher but it then dropped below the concentration of the nanocrystal sample. Accordingly, examples of higher apparent solubility values related to nanocrystals can be found, but still this clear benefit reach with nanocrystals are mostly left without consideration in nanocrystal applications.

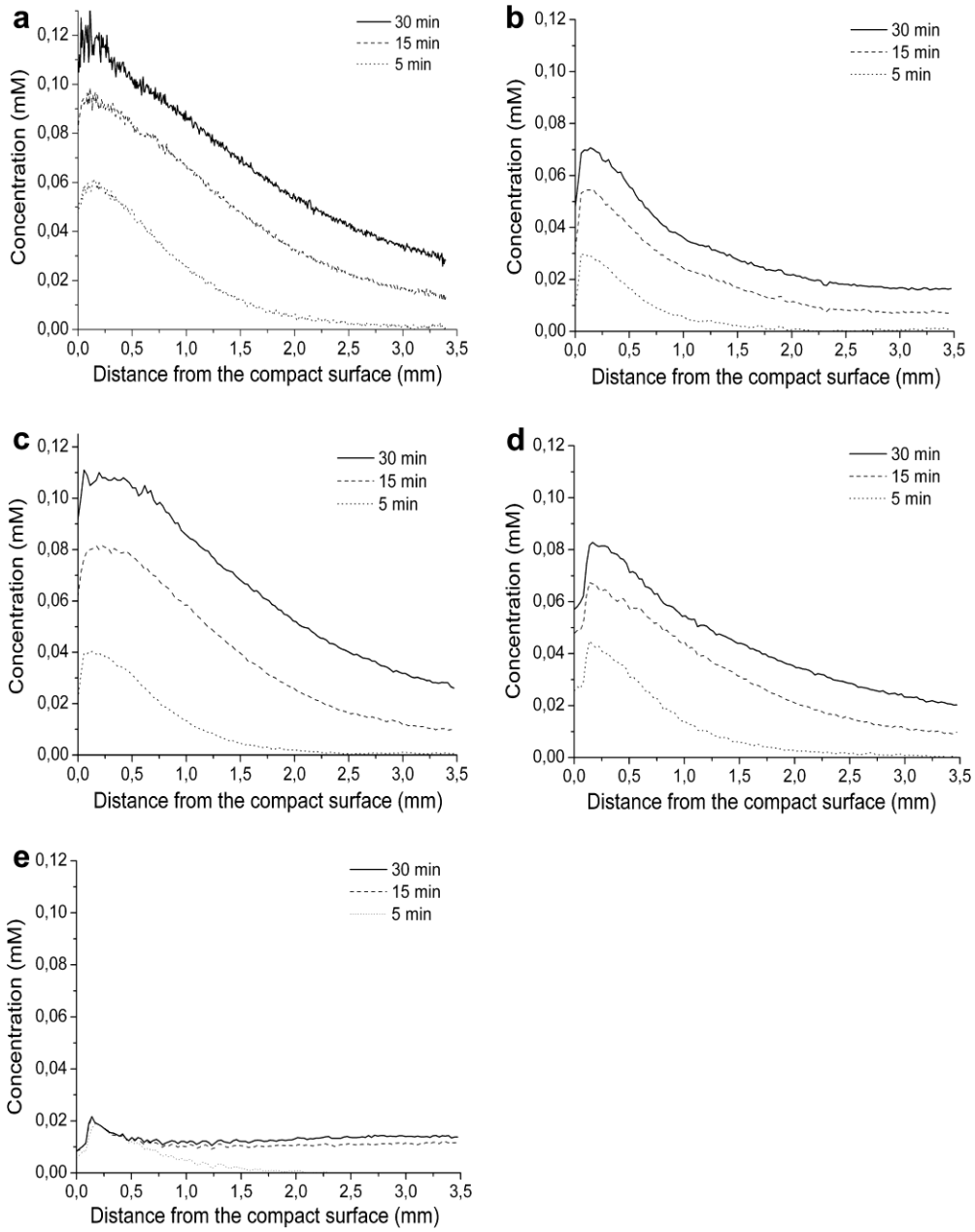

Figure 1. Apparent concentration-distance profiles from UV imaging of indomethacin nanocrystals (particle size 580 nm) and micron sized particles with two different stabilizers (poloxamers F68 and F127) as well as bulk indomethacin at 5, 15 and 30 min time points: (A) F68 stabilized nanocrystals, (B) F68 stabilized micron sized particles, (C) F127 stabilized nanocrystals, (D) F127 stabilized micron sized particles and (E) bulk indomethacin. (Reprinted from Sarnes et al., 2013 with permission from Elsevier).

The challenge in benefitting supersaturation is how to maintain it until the successful drug permeation. For example, in GI tract the pH changes may induce fast precipitation, like was the case with itraconazole nanocrystals produced by wet milling (Sarnes et al., 2014). Dried nanocrystals were packed in capsules, and *in vivo* tests were performed with rats. Though *in vitro* itraconazole nanocrystals showed superior dissolution rate as compared to Sporanox® granules, the *in vivo* bioavailability was higher with marketed

product. The problem with drug nanocrystals was that fast dissolution was followed by rapid transition of drug solution to intestine, where precipitation of itraconazole took place: as a basic compound, the solubility of itraconazole is appr. 250-times higher in stomach (lower pH) as compared to intestine (higher pH). However, when itraconazole nanocrystals were bind to nanofibrillar cellulose matrix, increased dissolution rate *in vitro* was also seen in enhanced *in vivo* performance of the drug: AUC-value with nanocrystalline formulation was 1.3 times higher as compared to marketed Sporanox® granules (Figure 2; Valo et al., 2011). Accordingly, the formulation was in key role in order to reach IVIVC.

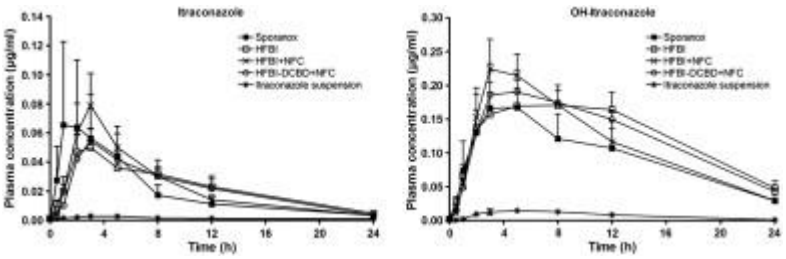

Figure 2. Plasma concentration profiles of itraconazole and OH-itraconazole in rats after oral administration of Sporanox® granules, three nanocrystalline formulations (HFBI, HFBI + NFC, HFBI-DCBD + NFC) and itraconazole microsuspension. (Reprinted from Valo et al., 2011, with permission from Elsevier.)

Another formulation factor, which should be taken into account is selection of stabilizer for drug nanocrystals. As already discussed, dissolution from drug nanocrystals can lead to supersaturated state. Also, it is well known that some polymers, like hydroxypropyl methylcellulose (HPMC), hydroxypropyl methylcellulose acetate succinate (HPMCAS), poly(vinyl pyrrolidone) (PVP), and Soluplus®, are able to prohibit drug crystallization from supersaturated solutions and hence capable of maintaining the system in highly concentrated state (Van Speybroeck et al., 2010; Chauhan et al., 2014; Ueda et al., 2014; Surwase et al., 2015, Figure 3); molecular level interactions between the polymer and the drug has been shown to determine the efficiency of maintaining the level of supersaturation (Chauhan et al., 2014; Ueda et al., 2014). Same above mentioned polymers has been utilized also as stabilizers for drug nanocrystals (Tuomela et al., 2014). In our preliminary studies we have shown same kind of crystallization prohibiting effect of HPMC and PVP in nanocrystalline systems (unpublished data), but with nanocrystals the impact of polymers still needs more studies in order to fully utilize the benefits of these polymers properly.

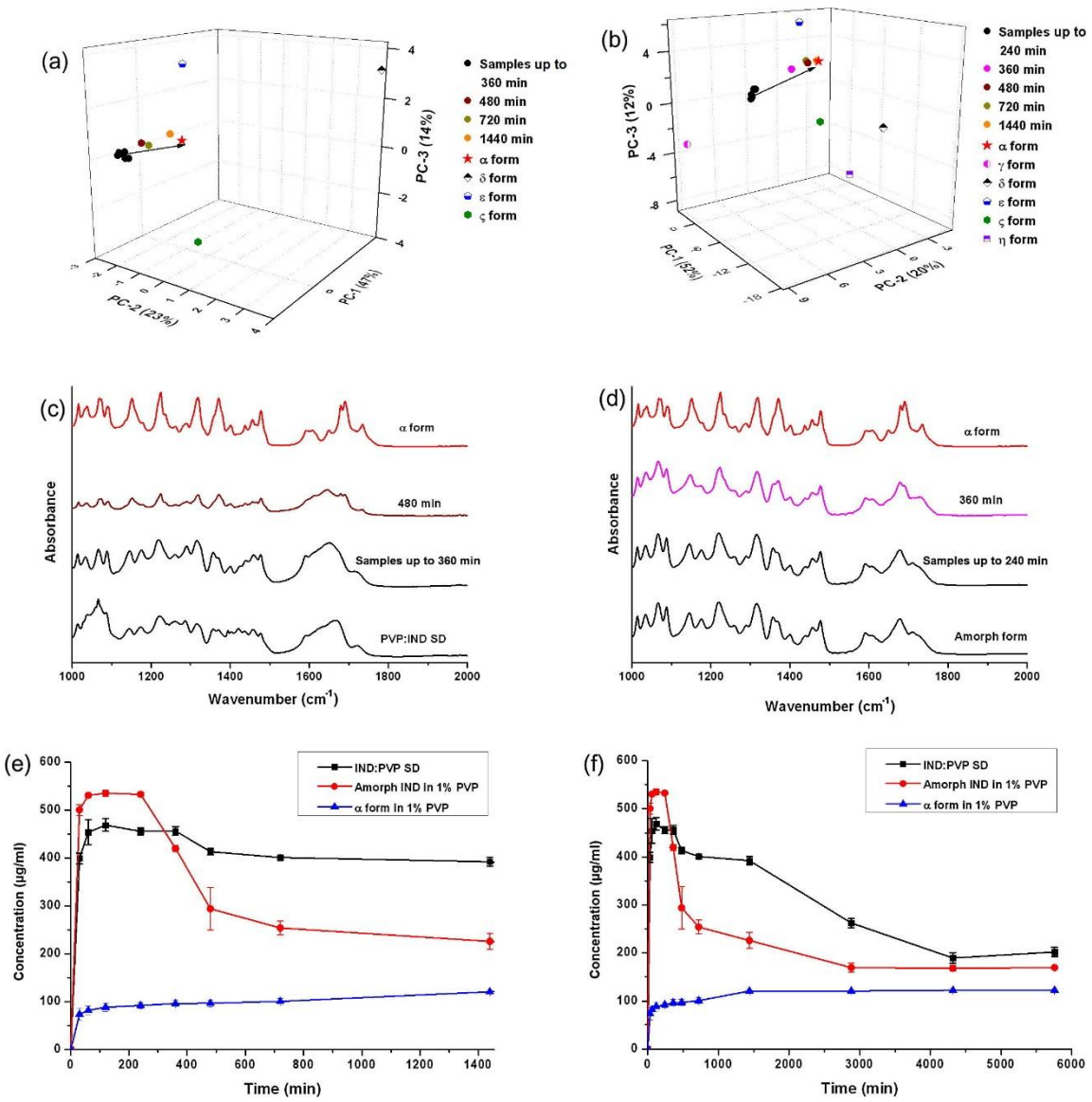

Figure 3. Impact of PVP on maintaining the supersaturated state of amorphous indomethacin for indomethacin-PVP solid dispersion (Figures a,c,e) and for amorphous indomethacin dissolved in aqueous PVP solution (Figures b, d, f) in pH 5.5 at 25 °C. Figures a and b: PCA scores plots of the IR spectra of indomethacin samples (arrows indicating the path of crystallization); Figures c and d: IR spectras of indomethacin samples; and Figure e and f: concentration-time profiles showing the maintenance of supersaturated state in the presence of PVP. (Reprinted from Surwase et al., 2015 with permission from Elsevier.)

2 Formation of drug nanocrystals

There are two approaches to make drug nanocrystals: i) top-down approach, where nanosized particles are produced by decreasing the particle size of bulk drug in a liquid suspension, for example by different kinds of milling or homogenization techniques (Keck and Müller, 2006; Peltonen and Hirvonen, 2010; Laaksonen et

al., 2011; Müller et al., 2011b; Möschwitzer, 2013), and ii) bottom-up approach, where the nanosized particles are built molecule by molecule by precipitation (Valo et al., 2011 and 2013; Wang et al. 2012). Most of the commercial pharmaceutical products are produced by top-down methods, mostly by milling, because in these techniques the process repeatability is at a high level and changes in scaling are considerably easy to perform (Chin et al., 2014). Process yield varies depending on the process and process type, and in the literature there are not many reports related to process yield. For example, liquid atomization based processes the yield can be very low, and material is lost on the atomization chamber walls. In milling processes, if the process is batch process, the yield can be considerably high, but again, material can be lost on the surfaces of the beads and the vessel.

2.1 Physical properties of drug nanocrystals

All the production techniques produce solid drug cores surrounded by a stabilizer layer, but depending on the process, certain properties, like particle shape, size, porosity and level of crystallinity, may be altered depending on the selected process and process parameters. Selection of stabilizer should be based on the drug properties, but it is also good to be aware of that many common stabilizers have some drug transport influencing activities. For example, many surfactants, like polysorbates, are able to open up tight junctions (Deli, 2009) and poloxamers and polysorbates are known to have effects on intestinal P-gp activity (Thakkar and Desai, 2015).

When thinking about the particle shape, milling for example typically produces edged particle shapes (Liu et al., 2011), while antisolvent precipitation or liquid atomization can lead to almost spherical particles (Valo et al., 2011). Also the raw material affects the particle shape: milling of itraconazole produced needle shaped particles while indomethacin formed oval particles (Liu et al., 2011). In the bottom-up techniques particle sizes below 100 nm are easily reached (Valo et al., 2011), but also modern high energy milling set-ups can lead to particle size fractions around 100 nm (Bujnakova et al., 2015a and 2015b; Li et al., 2015). More porous particles can be formed with liquid atomization based techniques, and with these techniques also amorphous material can be produced (Wang et al., 2012). Formation of amorphous material increases the solubility, but it also creates stability problems where uncontrolled crystallization is possible.

2.2 Quality by Design (QbD) approach for production of drug nanocrystals

All the listed properties are important to be aware of and to control because they can have an impact on dissolution, solubility and also cellular interactions *in vivo* (Liu et al., 2013; Ma et al., 2013; Sarnes et al., 2013; Shang et al., 2014), especially, when thinking of Quality by Design (QbD) approach for production of nanosuspensions (Ghosh et al., 2012; Peltonen and Strachan, 2015; Kassem et al., 2017; Li et al., 2017;

Soliman et al., 2017). QbD approach for nanosuspension production can be divided into three phases: i) selection of stabilizer(s) and production method based on Quality Target Product Profile (QTPP), ii) establishing Critical Quality Attributes (CQAs), and iii) formation of a design space based on Design of Experiments (DoE) (Li et al., 2017). CQAs can be for example particle size, shape, solubility or stability (Peltonen and Strachan, 2015). Critical process parameters (CPPs) aid in process controlling in order to find suitable tools for process control purposes during manufacturing, while proper design space ensures repeatable product performance batch after batch. For example, Afolabi et al. (2014) studied with the aid of microhydrodynamic model the effect of stirrer speed, bead concentration and drug loading on the bead-bead collisions and particle breakage kinetics during the wet milling process. They found out that increase in stirrer speed or bead concentration led to faster breakage via higher specific energy and milling intensity factor while increase in drug loading had opposite effect.

2.3 Down-scaling and up-scaling of nanocrystallization processes

Up-scaling and down-scaling are possible with some nanocrystallization techniques and mostly they have been studied with milling setups (Van Eerdenbrugh et al., 2009; Singare et al., 2010; Niwa et al., 2011; Ghosh et al., 2012; Tuomela et al., 2015). In milling aqueous drug-stabilizer suspension together with milling medium is agitated and scaling changes are considerably easy to perform. The total energy input and particle size reduction kinetics determine the final particle properties, and this can cause differences in final product properties (Date and Patravale, 2004; Bilgili and Afolabi, 2012; Afolabi et al., 2014; Tuomela et al., 2015). Yuminoki *et al.* (2016) used rotation/revolution mixer for media milling (Takatsuka et al., 2009; Yuminoki et al., 2016). The smallest batches were 100 mg of drug material. Later, process was scaled up to 1 kg batch size. Specific collisional energy was calculated by a theoretical equation modified for wet milling. Calculations showed that the relative centrifugal acceleration of revolution (straightly related to radius of the revolution and the number of revolutions per minutes) and drug concentration in the suspension where most important process variables: when these factors where identical, different scaling produced similar particle size fractions. Other successful scaling up case examples are: SmartCrystal combination process from laboratory to pilot scale (Shaal et al., 2010), combination process with static mixing and spray drying to continuous large-scale production (Hu et a., 2011), and precipitation followed by homogenization for large scale production (Quan et al., 2011).

Van Eerdenbrugh *et al.* (2008) performed milling in 96-well plate with 10 mg of drug: in screening tests the drug amount is enough for a thorough physicochemical characterization. Seven drugs were milled successfully, when the drug suspension together with milling pearls were put into the wells and the 96-well plate was agitated in an orbital shaker. Instead of milling particle breakage can be reached via acoustic mixing (Leung et al., 2014). In this technique, the drug suspension together with milling pearls are mixed in an

acoustic mixer. For screening purposes, the mixing can be performed in 96-well plate with only 2 mg of drug per well. Still another possibility is to put the suspension together with the milling pearls into small vials, which are packed inside of milling vessel. All the above mentioned protocols are suitable for preclinical screening purposes, but extra care should be taken with wearing of the well plate/vials, which leads contamination. In milling it is important that the milling pearls and vessel are from the same material, and softer well plate/vial material are vulnerable for erosion during milling process as well.

During high pressure homogenization, suspension is forced through a narrow homogenization gap, which limits the scaling down possibilities. However, equipment with 3.5 ml sample volume has been tested (Müller et al., 2001), and in laboratory scale the equipment can be used in discontinuos mode, which lowers the required sample amount (Grau et al., 2000).

2.4 Top-down nanocrystallization techniques

In top-down methods the particle size diminishing is based on mechanical attrition or high pressure collisions, and these can induce contamination due to the wearing and tearing of the equipment (Juhnke et al., 2012; Li et al., 2015). Process parameters like bead size/bead material, stirrer speed and energy input affect the level of contamination. With the same bead material, level of contamination can be minimized by shortening the process time and lowering the bead contact pressure by using smaller bead sizes. Other drawbacks of the top-down techniques are high energy consumption, particularly if the process times are long; however, today especially in milling the process times can be considerably shorter due to the more efficient milling equipment which lowers the overall energy consumption (Liu et al., 2011).

Milling and high pressure homogenization are performed in suspension. Mostly the suspension medium is water, but also oils or PEGs can be used (Keck and Müller, 2006; Al-Kassas et al., 2017). Possibility to avoid organic solvents has made these techniques environmental friendly (Chin et al., 2014; Peltonen et al., 2014). Presence of water also protects the contents against formation of amorphous material, because water enhances molecular mobility and lowers the glass transition temperature (Sharma et al., 2009). After milling the drug is typically in crystal form, although polymorphic changes are possible (Müller et al., 2001; Liu et al., 2011). High pressure homogenization may induce lowering of crystallinity, but also here the presence of water stabilizes the drug crystals (Müller et al., 2001; Sharma et al., 2009; Homayouni et al., 2014; Soliman et al., 2017).

Technically high pressure homogenization (HPH) can be separated in two different approaches: i) jet streaming (microfluidizer, IDD-PTM, insoluble drug delivery microparticle technology) and ii) piston-gap homogenization. In jet streaming high energy suspension flows collide in a microfluidizer, while in piston-gap

type homogenizer drug suspension is forced with high pressure through a narrow gap (Keck and Müller, 2006). Piston gap homogenization can further be divided in homogenization in water (Dissocubes®) (Müller et al., 2011a) and in non-aqueous media, like PEG (Nanopure®) (Radtke and Müller, 2001; Salazar et al., 2014).

2.5 Bottom-up nanocrystallization techniques

In bottom-up processes the drug nanocrystals are formed via precipitation/crystallization from a supersaturated solution. Bottom-up techniques have been studied a lot in laboratory scale, but scaling up is often problematic. Challenges are faced also due to difficulties in controlling the particle size growing, finding a suitable solvent/antisolvent combination, and the volume and demanding removing process of solvents. Especially solvent/antisolvent process mostly requires utilization of organic solvents due to poor solubility properties of drug materials.

Most traditional way is to induce precipitation via antisolvent addition, but also supercritical fluids, solvent removal or liquid atomization based techniques have been used (Valo et al., 2011; Sinha et al., 2013a; Valo et al., 2013; Sahu and Das, 2014). Especially liquid atomization techniques may produce amorphous materials due to the extremely fast solvent removal process, which can cause stability problems afterwards when amorphous drug starts to crystallize (Sinha et al., 2013a; Homayouni et al., 2014; Soliman et al., 2017).

2.6 Combination nanocrystallization methods

If the end product is not reaching the required CQAs in a single process, combination techniques can be utilized. Combination techniques are two step processes, which include i) pre-process step, for example pre-milling or precipitation and ii) high-energy top-down process (most often milling or high pressure homogenization) (Sinha et al., 2013b; Zong et al., 2017). Benefits of combination methods are that with them often even smaller particle sizes can be reached and avoidance of process related problems, like clogging of high pressure homogenizer, or shortening of the final top-down process time.

Though combination techniques can be beneficial when thinking of the end product properties or in avoiding process related challenges, more complicated process increases overall costs and complexity of the whole production process. Hence, combination techniques are never the first choice, and they should be selected only if clear benefits are reached by utilizing them.

First combination technique was antisolvent precipitation pre-process followed by high pressure homogenization (Nanoedge™; Möschwitzer, 2003). More recent are SmartCrystal® group of technologies where high pressure homogenization is combined with different pre-processes (Shegokar and Müller, 2010): H42 (spray-drying pre-process), H69 (precipitation pre-process), H96 (lyophilization pre-process) and CT

(media milling pre-process). More rarely studied combinations are for example antisolvent precipitation combined with ultrasonication (Soliman et al., 2017).

3 Drug nanocrystal based formulations

Nanocrystal formulations for drug delivery purposes have been increasingly popular in recent years (Lakshmi and Kumar, 2010; Arunkumar et al., 2009). As stated above in this review article, current drug discovery programs provide a high number of drug candidates showing high *in vitro* efficiencies, but actual clinical applications have often been restricted due to the poor aqueous solubility. Nanocrystals provide a potential way to overcome this problem and, thus, several successful, mostly orally administered products have already reached the market or are in the research pipelines (Junghanns and Müller 2008, Bansal et al., 2012; Table 1). Due to the increased surface area a rapid *in vivo* dissolution, fast absorption and increased bioavailability of these kinds of drugs have been reached. Administration of drug nanocrystals may take place via different drug delivery routes. Oral drug delivery route is the most popular and convenient and oral solid dosage forms of nanocrystals are usually preferred for commercialization. Sirolimus (Rapamune®) was the first nanocrystalline drug on the market in 2000, soon followed by other orally administered nanocrystalline formulations like megestrol acetate (Megace®, 2001), aprepitant (Emend®, 2003) and fenofibrate (Tricor®, 2004) (Bobo et al., 2016).

Table I. Examples of nanocrystalline products on the market approved by the US FDA. Modified from Bobo et al. (2016) and Gao et al. (2013).

Drug	Indication	Special notes	Process
Aprepitant	Antiemetic	Faster absorption and higher bioavailability	Milling
Fenofibrate	Hyperlipidemia	Higher bioavailability, easier administration	Milling
Sirolimus	Immunosupressant	Higher bioavailability	Milling
Megestrol acetate	Anti-anorexic	Reduced dosing	Milling
Morphine sulfate	Psychostimulant	Higher drug loading and bioavailability, extended release	Milling
Dexamethyl-phenidate HCl	Psychostimulant	Higher drug loading and bioavailability	Milling
Methylphyenidate HCl	Psychostimulant	Higher drug loading and bioavailability	Milling
Tizanidine HCl	Muscle relaxant	Higher drug loading and bioavailability	Milling
Calcium phosphate	Bone substitute	Mimics bone structure allowing cell adhesion and growth	NanOss™
Palperidone palmitate	Schizophrenia	Allows slow release of injectable low solubility drug	Milling, HPH

Drug development in mind, the size-controlled nanocrystals of a drug under development are often converted into dry powders, which are further formulated into dosage forms: tablets, capsules, pellets or liquid nanocrystal suspensions. Gao *et al.* (2012) listed the benefits of nanocrystalline and nanoparticulate dosage forms in oral drug delivery: enhanced oral bioavailability due to the improved drug dissolution/solubility, reduced fasted/fed state variation in drug absorption, potentially improved transcellular uptake or prolonged mucoadhesion of the nanoparticles, and improved safety profiles of the nanocrystal formulations. The mucoadhesive and gastroretentive properties of coated (stability-improved)

nanoparticles can be modified, for example, with hydrophobin proteins (Sarparanta et al., 2012). Upon entry of the nanoparticles from the stomach into the intestines, it was observed that hydrophobin-protein coated nanoparticles were retained in rat stomach up to three hours after administration, whereas corresponding uncoated nanoparticles were released significantly faster in the same conditions.

Despite the benefits above and the relative ease of administration, nanocrystals provide special challenges in the design of oral solid drug formulations. Tuomela *et al.* (2015) screened comprehensively powder and tablet compositions of indomethacin and itraconazole nanocrystals in order to find out optimal properties for the tableting conditions and tablet formulations. As such, the mere presence of nanocrystals in the composition improved the compressibility of tablets. The smaller the particle size was, the more contact surfaces there were providing potential inter-particle bonds that resulted in increased hardness/crushing strength of the tablets. When the nanocrystals were processed into granules before tableting, a further decrease in the required compression force was detected (Tuomela et al., 2015). Disintegration testing of the tablets revealed changes in the texture and inner structures of the tablets: the less was the amount of drug nanocrystals in the formulation, the more porous was the structure formed. Disintegration times correlated also well with the crushing strength values of the tablets. Composition-wise, it was found out, at least in the cases of indomethacin and itraconazole, that the optimal amount of freeze-dried nanocrystals in the tablet composition was about 40% of the total mass (corresponding to theoretical drug loading of 22-29%), in which cases the improved dissolution profiles and disintegration times of the corresponding nanocrystal tablets were still maintained.

After oral delivery, development of parenterally administered nanocrystal formulations is the second most popular approach. Intravenous, intramuscular and subcutaneous delivery routes provide a quick (i.v.) or potentially retarded (i.m.) onset of action, rapid reach of different body parts and organs with concomitant potential for drug targeting, and reduced dosage need of the drug. These routes are beneficial for drugs undergoing first-pass metabolism and drugs that are not absorbed or are degraded/irritating in the GI tract. Nanoparticles have potential as novel intravascular formulations for both diagnostic (imaging) and therapeutic purposes (drug delivery), or even combination of these (theranostics) (Ahmed et al., 2012). Successful parenteral nanoformulation delivery requests the drug to be able to target in specific tissues and cell types and escape from the reticuloendothelial system (Åkerman et al., 2002). Parenteral administration of nanocrystals has certain advantages: administration of poorly soluble drugs without using high concentrations of toxic co-solvents, improved therapeutic effect of the drug, and targeted drug delivery to macrophages. Obvious drawback of this delivery route is the invasiveness of the mode of administration and the associated poor patient compliance.

Fuhrmann *et al.* (2014) discuss thoroughly the benefits and difficulties in the *in vitro* - *in vivo* correlation of injectable, non-targeted and targeted nanocrystals. As small (< 400 nm) and adjustable systems, the intravenously injected nanocrystals are able to extravasate from the blood through the leaky endothelium and accumulate in, for example, tumor tissue via the enhanced permeation and retention (EPR) effect (Matsumura and Maeda, 1986). In addition to this passive targeting phenomenon, some of the stabilizers can be functionalized with targeting/internalizing ligands to promote active tumor accumulation or uptake, respectively. These vital stabilizers, for example polymers or surfactants, typically stabilize nanocrystals by adsorbing to the surfaces and provide steric (e.g., poloxamers, cellulose derivatives) or electrostatic (e.g., sodium dodecyl sulfate, Tween®) barriers to aggregation (Peltonen and Hirvonen, 2008; Fuhrmann et al., 2014). As only a small amount of stabilizing agent is typically required to mask the nanocrystals and prevent their aggregation, drug contents of typically 50 to 90% (wt) are reached, which is clearly higher than with some other nanocarrier systems. As described in more detail in this review, nanocrystals exhibit a characteristic nonlinear increase of kinetic solubility upon miniaturization that is described by the Ostwald–Freundlich equation (Chapter 1.2). This increased rate of dissolution is generally utilized for the non-targeted delivery. A drawback in targeted i.v. nanocrystal delivery is that the enhanced dissolution interferes and reduces the efficacy of targeted nanocrystalline drug delivery: increased drug delivery off the target, reduced circulation time of the nanosystems, and potential sub-standard efficacy and utilization of the stabilizing agents and/or targeting agents.

3.1 Development of nanocrystalline cancer drug formulations

In order to increase the cancer drug deposition in cancerous tissues, the research group of Professor Leroux, among others, has applied different strategies for nanocrystals delivery and action: instead of utilizing the fast and high dissolution, they have intensively tested polymer-coated paclitaxel nanocrystals with slow dissolution and retarded drug release properties (Polomska et al., 2017). Successful delivery of the drug to the site of disease requests that the nanocrystals should deposit a significant amount of the cancer drug to the right place in order to improve the treatment efficacy, although at the same time the formulation should minimize the potential drug-associated, and also the excipient-associated, side effects. In this respect the small size and high-energy nanocrystals surfaces lead to a too rapid dissolution and administration, which may oftentimes reduce the local accumulation of the drug at the exact sites of cancerous tissue(s). In the case of paclitaxel nanoparticles, this might lead to markedly higher risk of hypersensitivity reactions and higher incidence of neuroptenia. Attempts to extend and prolong the delivery and improve the relatively low accumulation of anticancer drug nanocrystals has been tested by polyelectrolyte multilayers (layer-by-layer technology; Polomska et al., 2017), by PEGylated nanocages as non-sheddable stabilizers for drug

nanocrystals (Fuhrmann et al., 2012), and by redox-responsive stabilizers for drug nanocrystals (Fuhrmann et al., 2013).

Also other groups have studied the extended and targeted delivery of the cancer drug paclitaxel. Deng et al. (2010) studied the stabilization method on paclitaxel-Pluronic F127 nanocrystals (Figure 4). Increased drug dosing was expected to result in improved antitumor activity of paclitaxel without the incidence of acute toxicity. Desorption experiments of Pluronic F127 stabilizer showed different surfactant adsorption affinities to the paclitaxel nanocrystal surfaces above and below the critical micelle concentration (CMC) of the polymer. Below the CMC the monomers were bound to the nanocrystal surface with high affinity, but above the CMC the low affinity surfactant aggregates were removed rapidly from the nanocrystal surfaces upon dilution. The overall conclusion in this study was that in order to improve the stability of nanocrystals, re-nanonization by incubation–sonication procedure should be used to disrupt the preferred crystal growth patterns of paclitaxel (Deng et al., 2010).

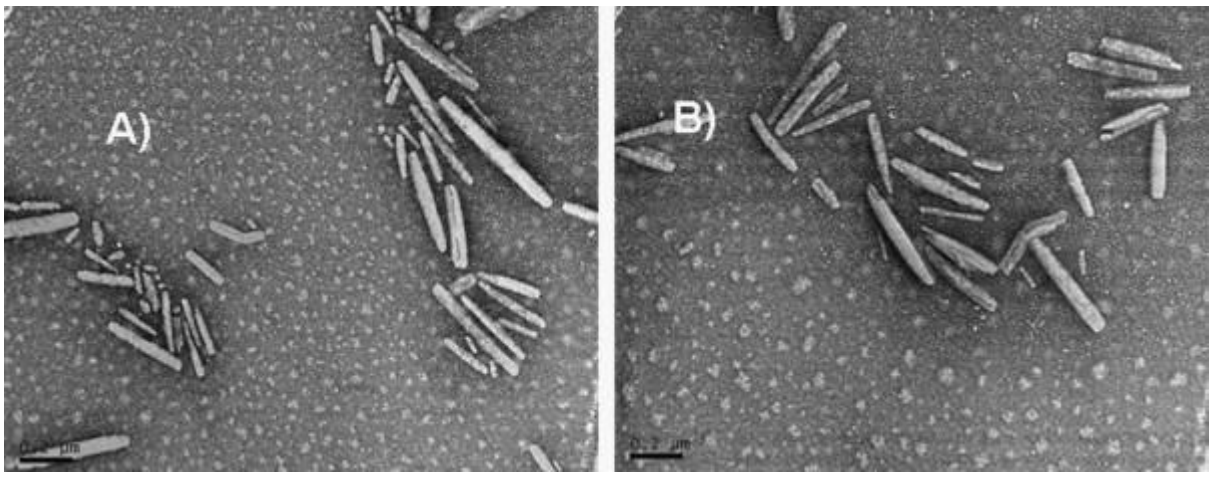

Figure 4. TEM figures of F127 stabilized paclitaxel nanocrystals. Drug surfactant ratio (A) drug:surfactant ratio 1:10 (w/w) and (B) 1:20 (w/w). Scale bar is 200 nm. (Reprinted from Deng et al., 2010 with permission from Elsevier).

Lu *et al.* (2014) prepared and evaluated paclitaxel nanocrystal formulations by stabilizing them non-covalently with a serum protein transferrin. In addition to transferrin, also other serum proteins including albumin and immunoglobulin G were evaluated with respect to the stabilizing effect. *In vivo* antitumor efficacy studies were conducted in mice that had been inoculated with drug containing KB cells. The results demonstrated significantly higher tumor inhibition rate (45%) for the paclitaxel-transferrin formulation compared to the paclitaxel nanocrystal treatment alone (29% inhibition) (Figure 5). It is to be noted here that commercial Taxol® formulation showed higher antitumor activity in mice than the paclitaxel-transferrin study formulations, reaching a 93% tumor inhibition rate (Lu et al., 2014). On the other hand, the paclitaxel-

transferrin formulations showed lower levels of toxicity, which was indicated by a steady increase in body weight of the mice during the cancer treatment period. In comparison, treatment with Taxol® resulted in toxicity related problems as the body weight of the mice was decreased.

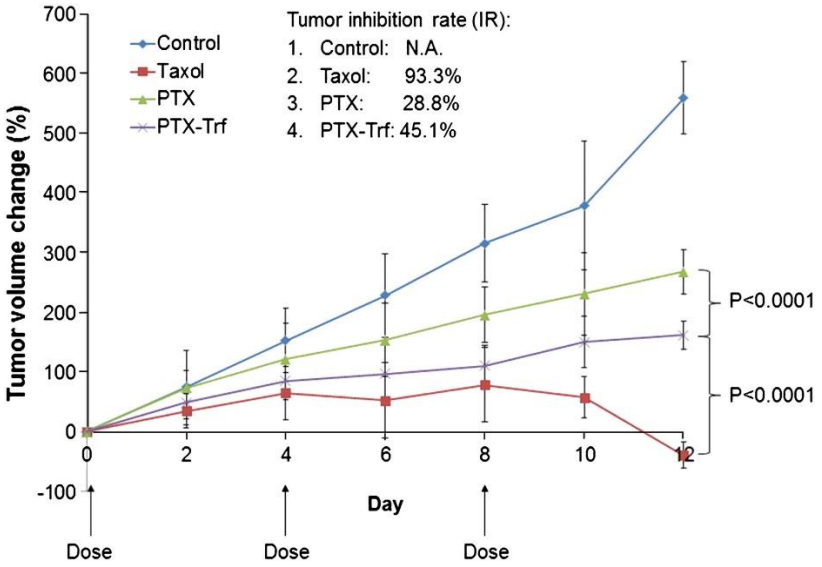

Figure 5. In vivo antitumor efficacy of paclitaxel formulations in mice: curves from top to down: Control, PTX paclitaxel nanocrystal suspension, PTX-Trf paclitaxel transferrin nanocrystal suspension and Taxol®. (Reprinted from Lu et al., 2014 with permission from Elsevier).

Professor Torchilin's group prepared stable nanocrystal colloids of poorly soluble cancer drugs paclitaxel and tamoxifen with very high drug loadings (up to 90% wt) utilizing the layer-by-layer technology, alternating the adsorption of oppositely charged polyelectrolytes on the surface of drug nanocrystals produced by ultrasonication of larger drug crystals (Agarwal et al., 2008). Such polymeric coatings prevent the aggregation of drug nanocrystals and create stable polymeric shells on their surface. Drug release rates of the cancer drug nanocrystals can be controlled by assembling multilayer shells with variable shell densities and thicknesses. Also here various specific targeting ligands could be rather easily attached to the surfaces of the nanosystems by using polymers with free reactive groups on the outer coating, e.g., free amino groups. Shutava *et al.* (2012) prepared 150-200 nm nanocapsules containing 60–70% (wt) of poorly soluble paclitaxel and camptothecin, again with the layer-by-layer assembly of the drug nanocores, in solutions containing uncharged stabilizers. Concentrated colloids of the cancer drugs (3-5 mg/mL) were found out to remain stable in isotonic salt buffers. Nanocrystal aggregation during the layer-by-layer-assembly was prevented by using minimal amounts of low molecular weight block-copolymers of poly-L-lysine and poly-L-glutamic acid with polyethylene glycol (PEG) in combination with heparin and bovine serum albumin at each bilayer building step. The PEGylated nanosystems presented high colloidal stability in PBS buffer and increased protein adhesion resistance.

SN-38 (7-Ethyl-10-hydroxycamptothecin) is another potent broad-spectrum antitumor drug, an irinotecan derivative. As the compound is poorly soluble and instable (with a labile active lactone ring), the clinical use of this compound has been compromised. Nanocrystal formulations have, therefore, been attempted to solve these problems and evaluate the true antitumor effect of SN-38 in vitro and in vivo (Chen et al., 2017). Nanocrystals with clearly different particle sizes were prepared, SN-38/NCs-A and SN-38/NCs-B, with mean diameters of 230 and 800 nm, respectively. Dissolution and release rate results in the case of SN-38/NCs-A were significantly faster than with SN-38/NCs-B. Accordingly, enhanced intracellular accumulation of SN-38/NCs-A was observed in HT1080 cells compared to that of SN-38/NCs-B nanocrystals and SN-38 solution. Moreover, the SN-38/NCs-A nanocrystals provided a higher bioavailability and significant inhibition of tumor growth compared to the SN-38 solution and SN-38/NCs-B nanocrystals in vivo after intravenous injection (Chen et al., 2017). The tissue distribution study in tumor-bearing mice showed that the nanocrystals could markedly improve the drug accumulation in tumor tissues by, presumably, the EPR effect when compared to SN-38 solution. The amount of SN-38 in tumors of after the treatment with SN-38/NCs-A nanocrystals was clearly higher than after the delivery of SN-38/NCs-B nanocrystals.

As stated repeatedly above, one interesting way to slow down the dissolution and improve the (targeted) delivery of anticancer nanocrystals is the utilization of layer-by-layer assemblies of polyelectrolytes around the nanocrystals. As shown in the above study examples, this has been proven to be a successful strategy at least *in vitro*. However, *in vivo* nanoparticles with charged surfaces are highly susceptible to opsonization and clearance by the mononuclear phagocyte system, leading to short biological half-lives and fast accumulation in the liver and spleen (Sarparanta et al., 2012). This fast clearance obviously diminishes significantly the tumor accumulation of “normal” nanocrystals *in vivo*. Flexible hydrophilic polymers like poly(ethylene glycol) on top of the multilayers are thus warranted in order to sterically hinder the adsorption of plasma proteins on the nanosystems (Polomska et al., 2017). As of today, little is still known about the stability of PEGylated polyelectrolyte-coated drug nanocrystals in the complex environment of blood circulation and tissue compartments, and also about the circulation times or drug biodistribution profiles of these nanosystems *in vivo*.

3.2 Orally administered nanocrystal formulations

Despite the numerous success stories (Table 1), formulation and delivery of nanocrystalline dispersions, tablets and other solid formulations via the GI-tract are often not straightforward processes (Gao et al., 2012). The impact of physiological factors like the variation of pH and peristalsis towards the nanocrystals are not simple to predict. The acidic nature of a drug affects strongly to the rate and extent of nanocrystal dissolution in the GIT. For example, in the cases of indomethacin and itraconazole the fast *in vitro* dissolution may not be maintained in the gut/intestines *in vivo*, leading to potentially uncontrolled drug precipitation

during the transit in the GI-tract (Sarnes et al., 2014, Figure 6). In the GI fluids, the disintegration of the nanoformulation leads to the formation of nanocrystal dispersion. Stabilizer molecules attached on the surface of nanocrystals will offer again ionic or steric repulsion, given that they are not affected by the gut/intestines environments (Peltonen and Hirvonen, 2008). Just like with the injectable nanocrystal formulations, the most orally relevant nanocrystal stabilizers are found in the groups of polymeric and non-ionic surfactants, such as poloxamers or polysorbate (Tween®) 80, as these stabilizers provide effective steric repulsion in GI fluids, given that the amount of stabilizers is adequate (Lai et al., 2014). Generally, again, ionic stabilizers like NaCMC and SDS, are effective in aqueous environment, but often the ionized state is not maintained in dry nanocrystalline powder material, thus making them less effective. Furthermore, ionic stabilizers are sensitive to pH changes and ionic strength when the dry powders are redispersed in the GI fluids.

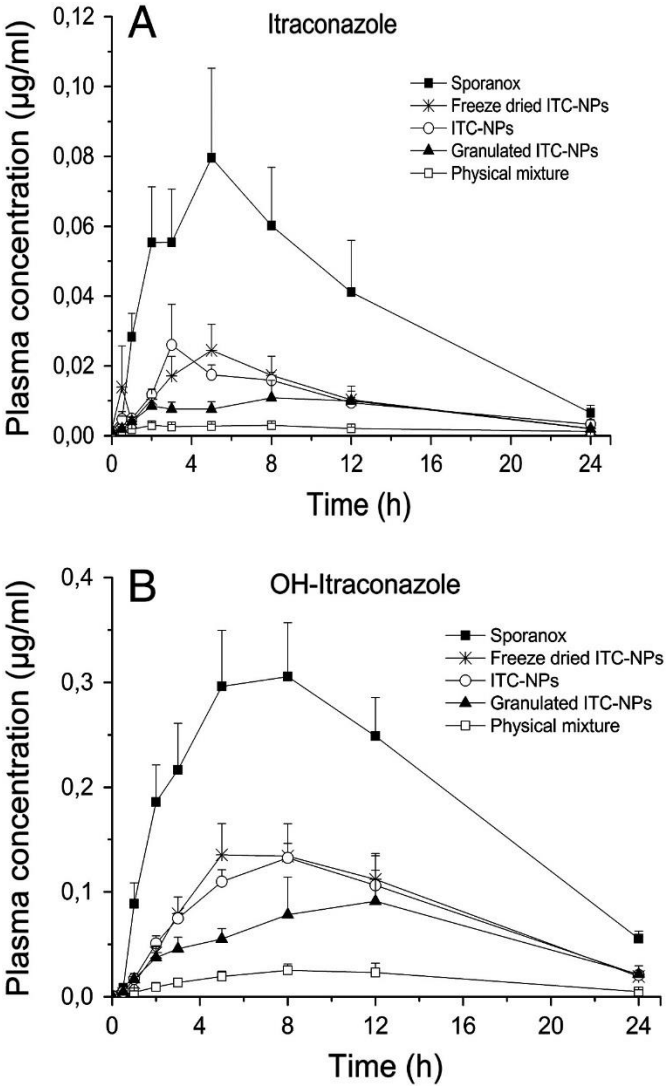

Figure 6. Bioavailability in rats of itraconazole and OH-itraconazole after per oral administration of nanocrystalline itraconazole formulations: nanocrystal suspension (ITC-NPs), freeze dried nanocrystals

(freeze dried ITC-NPs), granulated nanocrystals (granulated ITC-NPs), Sporanox® granules and physical mixture (mean ± sem, n = 5–6). (Reprinted from Sarnes et al., 2014 with permission from Elsevier).

Rahim *et al.* (2017) attempted to enhance the dissolution rate, oral bioavailability and analgesic potential of aceclofenac nanocrystals in Swiss albino rabbits. The nanocrystal suspensions were produced by a precipitation–ultrasonication method with hydroxypropyl methylcellulose, polyvinylpyrrolidone and sodium dodecyl sulfate as stabilizers/excipients. Saturation solubility of aceclofenac nanocrystals was increased 2.6- and 4.5-fold compared with unprocessed API in stabilizer solution and with totally unprocessed drug, respectively. As expected, the dissolution rate of the aceclofenac nanocrystals was substantially enhanced *in vitro*, and also the *in vivo* studies of stabilized aceclofenac nanocrystal suspension showed increased C_{max} (4.98- and 2.80-fold) and $AUC_{0\rightarrow 24\ h}$ (3.88- and 2.10-fold) values of the nanocrystal formulations when compared with the unprocessed drug and the currently marketed formulation of aceclofenac, respectively. The improved antinociceptive (pain receptor relieving) activity of the aceclofenac nanocrystals was also shown at lower drug doses. The same group (Shah et al., 2016) developed nanocrystalline formulations of antimalarial drug artemether, another compound of poor solubility and consequently low bioavailability. "Smart nanocrystals" of artemether were produced using a wet milling technology resulting in mean artemether nanocrystal particle sizes of 160 nm. The saturation solubility of the artemether nanocrystals was substantially increased to 900 μg/mL, compared to the raw artemether solubility in water (145 μg/mL) and artemether microparticles in stabilizer solution (300 μg/mL). Results of *in vitro* studies showed significant antimalarial effect of artemether against Plasmodium falciparum and Plasmodium vivax cultures. The IC_{50} (median lethal oral dose) values of artemether nanocrystals were 28-54-fold lower than the IC_{50} values of unprocessed drug and 13-21-fold lower than the IC_{50} values of marketed artemether tablets, respectively. A 2 mg/kg dosing of artemether nanocrystals showed significantly higher (89%) reduction in parasitemia against Plasmodium vivax compared with unprocessed artemether (27%) or the marketed artemether tablets (45%) (Shah et al., 2016). An acute toxicity study in Swiss albino mice demonstrated that the LD_{50} value of artemether nanocrystals was between 1,500 mg/kg and 2,000 mg/kg when given orally.

Professor Rainer H. Müller and his research group has extensively studied also the oral drug delivery route, see for example Müller *et al.* (2001; 2006). The group has developed and optimized oral nanoformulations for cyclosporine A (2%) as solid lipid nanoparticles (SLN™, mean size 157 nm) and as nanocrystals (mean size 962 nm). The encapsulation of cyclosporine A in SLN was 96%, while the nanocrystals were composed of 100% of the drug. The blood profiles in young pigs after the oral administration revealed that for the drug nanocrystals most of the blood concentration values were low with high differences between the measuring time points and the tested animals. On the contrary, administration of cyclosporine-loaded SLN led to higher mean plasma profiles with low variations, while at the same time successfully avoided the potential side

effects by high blood concentrations, as was the case with the commercial microemulsion product of cyclosporine-A (Sandimmun®).

Overall, pharmaceutically relevant nanocrystal formulations have been widely studied and commercialized with improved solubility/dissolution properties of poorly soluble drug materials. Nanocrystals are often formed from 100% drugs covered by stabilizer layer(s) in relatively simple and efficient manufacturing processes.

Conclusions

Nanosizing is simple and straightforward way to improve solubility properties of poorly soluble drug materials, and often even very small changes in particle size are enough for acceptable product performance. The most important property of drug nanocrystals is increased dissolution rate due to the smaller particle size, but in nanoscale physical properties like solubility are also different from thermodynamic solubility value. These two important properties of drug nanocrystals can be utilized in order to reach higher bioavailability with nanocrystal formulations, and drug nanocrystals are one versatile option for improving solubility properties of BCS class II and in some cases also class IV drugs. There are already a lot of studies and marketed products with different formulations in various administration routes based on drug nanocrystals. In the future the research will be headed more on functional properties of the stabilizers utilized in drug nanocrystals, role of supersaturation and QbD approach in formulation design, and drug targeting applications for example in cancer therapeutics or theranostics through the attachment of protecting layers and targeting ligands on the surfaces of the nanosystems.

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Figure Captions:

Figure 1. Apparent concentration-distance profiles from UV imaging of indomethacin nanocrystals (particle size 580 nm) and micron sized particles with two different stabilizers (poloxamers F68 and F127) as well as bulk indomethacin at 5, 15 and 30 min time points: (A) F68 stabilized nanocrystals, (B) F68 stabilized micron sized particles, (C) F127 stabilized nanocrystals, (D) F127 stabilized micron sized particles and (E) bulk indomethacin. (Reprinted from Sarnes et al., 2013 with permission from Elsevier).

Figure 2. Plasma concentration profiles of itraconazole and OH-itraconazole in rats after oral administration of Sporanox® granules, three nanocrystalline formulations (HFBI, HFBI + NFC, HFBI-DCBD + NFC) and itraconazole microsuspension. (Reprinted from Valo et al., 2011, with permission from Elsevier.)

Figure 3. Impact of PVP on maintaining the supersaturated state of amorphous indomethacin for indomethacin-PVP solid dispersion (Figures a,c,e) and for amorphous indomethacin dissolved in aqueous PVP solution (Figures b, d, f) in pH 5.5 at 25 °C. Figures a and b: PCA scores plots of the IR spectra of indomethacin samples (arrows indicating the path of crystallization); Figures c and d: IR spectras of indomethacin samples; and Figure e and f: concentration-time profiles showing the maintenance of supersaturated state in the presence of PVP. (Reprinted from Surwase et al., 2015 with permission from Elsevier.)

Figure 4. TEM figures of F127 stabilized paclitaxel nanocrystals. Drug surfactant ratio (A) drug:surfactant ratio 1:10 (w/w) and (B) 1:20 (w/w). Scale bar is 200 nm. (Reprinted from Deng et al., 2010 with permission from Elsevier).

Figure 5. In vivo antitumor efficacy of paclitaxel formulations in mice: curves from top to down: Control, PTX paclitaxel nanosuspension, PTX-Trf paclitaxel transferrin nanosuspension and Taxol®. (Reprinted from Lu et al., 2014 with permission from Elsevier).

Figure 6. Bioavailability in rats of itraconazole and OH-itraconazole after per oral administration of nanocrystalline itraconazole formulations: nanosuspension (ITC-NPs), freeze dried nanosuspension (freeze dried ITC-NPs), granulated nanosuspension (granulated ITC-NPs), Sporanox® granules and physical mixture (mean ± sem, n = 5–6). (Reprinted from Sarnes et al., 2014 with permission from Elsevier).