Pharmaceutical nanocrystals: Production by wet milling and applications

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Teaser: This review outlines the advantages, stabilisation and production of drug nanocrystals with emphasis on wet milling. Covering their pharmaceutical applications, it reveals why nanocrystals are an industrially feasible formulation strategy.

Abstract

Nanocrystals are regarded as an important nanoformulation approach exhibiting the advantages of increased dissolution and saturation solubility with chemical stability and low toxicity. Nanocrystals are produced in the form of nanosuspension using top-down (e.g. wet milling, high pressure homogenization) and bottom-up methods (e.g. antisolvent precipitation). Wet milling is a scalable method applicable to drugs with different physicochemical and mechanical properties. Nanocrystalline-based formulations, either as liquid nanosuspensions or after downstream processing to solid dosage forms, have been developed as drug delivery systems for various routes of administration (i.e. oral, parenteral, pulmonary, ocular and dermal). In this review, we summarize and discuss features, preparation methods and therapeutic applications of pharmaceutical nanocrystals highlighting their universality as a formulation approach for poorly soluble drugs.

1. Introduction

The physicochemical properties of many new chemical entities (NCEs), which are developed as future drug candidates, are moving towards higher molecular weight and higher lipophilicity in the quest for biological selectivity and specificity [1]. These physicochemical properties often result in compounds with low aqueous solubility. Thus, many of the NCEs arising from high throughput screening and combinatorial chemistry methodologies (> 40%) suffer from poor solubility in aqueous media and some of them simultaneously in organic solvents [2]. The poor solubility of a compound is related with several biopharmaceutical problems. For example, in the case of oral administration, NCEs which possess limited solubility and dissolution rate in the digestive juice may display low bioavailability, high fed/fasted state variability, high interpatient variability, retarded onset of action, lack of dose proportionality and local irritation [3]. It is evident then, that the limitation of poor solubility which constitutes one of the main reasons for the discontinuation of development of NCEs, makes their formulation very challenging.

In the past, the pharmaceutical industry considered these compounds as highly risky development candidates. However, nowadays mainly due to their prevalence, '*industry consensus has shifted from an attitude of avoidance to one of acceptance and increasing research dedication is given to solving solubility challenges*' [4].

Several formulation strategies are currently used in order to improve the solubility, dissolution rate and subsequent bioavailability of drugs. These strategies include modifications of the drug properties on the molecular level (e.g. salt or prodrug

formation, use of co-solvents, complexation with cyclodextrins), the use of colloidal drug delivery systems (e.g. microemulsions, self-microemulsifying systems) or modifications of the drug properties on the particulate level (e.g. particle size reduction, amorphization) [5].

2. Nanoparticles in drug delivery

Nanotechnologies are considered one of the most prevalent improvement methods and have been used to overcome the problem of poor solubility and thus bioavailability, as well as to achieve targeted drug delivery.

Despite the importance of nanoparticles, there is no single definition of nanoparticles. This may be due to the highly multidisciplinary nature of nanotechnology. The term nanotechnology was first used by the scientist Norio Taniguchi in 1974, at the University of Tokyo in Japan, for any material in the nanometre size range [6].

According to the U.S. Food and Drug Administration (FDA), materials are classified as being in the nanoscale range if they have at least one dimension at the size range of approximately 1-100 nm. However, as many properties characteristic of the nanoscale (e.g. solubility, light scattering, surface effects) are predictable and continuous characteristics of the bulk materials [7], the definitions of "nanomaterial" based on size are often inconsistent and the upper end of the nanoscale at 100 nm is an arbitrary cut-off size [8]. Thus, the 100 nm limit is often considered constraining and according to a more inclusive definition: particles below 1000 nm in each dimension (submicron particles) are designated as nanoparticles [9]. The latter definition is applicable in the pharmaceutical field as

particle size in the nanometre range can lead to increased dissolution rate due to the increase in surface area and increased saturation solubility [10].

Various types of nanotherapeutics have been applied in drug delivery. The types of nanotherapeutics approved for oral or parenteral drug delivery in the EU market include liposomes, nanoemulsions, polymeric therapeutics, polymeric nanoparticles, virosomes, nanocomplexes and nanocrystals (Fig.1, [11])

3. Nanocrystals

Nanocrystals are nanosized drug particles. Nanocrystals are typically produced in the form of nanosuspensions, which are submicron (colloidal) dispersions of drug particles, stabilized by surfactants, polymers, or a mixture of both [12]. According to a stricter definition, a formulation should have a volume median diameter (D_{50}) below 1μm and a volume diameter 90% undersize (D_{90}) below 2.5 μm to be classified as a nanosuspension (Fig. 2, [13–15]). At this point, it should be noted that while dynamic light scattering is a common ensemble technique for the determination of the particle size of the nanosuspensions, it can lead to false assumptions regarding the particle size and should always be complemented with additional techniques like transmission electron microscopy [16,17]. The term nanocrystals, although implying the particles are in a crystalline state, which is true for most of the reported cases, has been extended to describe nanosized suspensions of partially crystalline [18] or even amorphous drugs formed due to changes from the crystalline to the amorphous form during processing [19,20]. In the strict sense, such an amorphous drug nanoparticle should not be called a nanocrystal. Recently, preparation of nanosized drug particles in the amorphous state is gaining momentum as the combination of size reduction with

amorphization has shown clear superiority for enhancing the dissolution rate and solubility of poorly soluble drugs. Various terms have been used for the description of nanoparticles in the amorphous state (e.g. ‘amorphous nanoparticles’ [21], ‘amorphous drug nanosuspensions’ [22] and even ‘nanosuspensions’ [18]).

Drug nanosuspensions have been suggested as a universal delivery approach for orally administered drug molecules that fall into class II (low solubility, high permeability) and class IV (low solubility, low permeability) of the Biopharmaceutics Classification system (BCS) [9,23]. Butler and Dressman [24] proposed the Developability Classification System (DCS) as a way to categorize compounds in a more bio-relevant manner. According to the DCS, which distinguishes between dissolution rate-limited (DCS Class IIa) and solubility-limited compounds (DCS Class IIb), the intrinsic solubility and the related intraluminal drug concentration for compounds belonging to Class IIb and IV are too low to achieve sufficient flux over the epithelial membrane. Hence, complexation or formulation approaches based on solid state modification may be preferable compared to nanocrystals for compounds belonging to DCS Class IIb and IV [25–27].

Yalkowsky and co-workers established the General Solubility Equation, in which the solubility of a compound is expressed as a function of the melting point and its lipophilicity (in form of octanol-water partition coefficient, log K_{ow}) [28]. Poorly soluble drugs are often referred to as "grease balls" and "brick dust" molecules. Specifically, "grease balls" represent highly lipophilic compounds (log $K_{ow} > 3$) which are poorly hydrated and their solubility is solvation limited while "brick

dust" compounds display lower lipophilicity and higher melting point (m.p. > 200 ^{o} C) and their solubility is limited by the strong intermolecular bonds within the crystal [29]. Brick dust molecules have been found to benefit from formulation approaches such as particle size reduction and amorphization while grease balls can be formulated as lipid-based formulations [30]. Thus, formulating drugs as nanocrystals should be mainly employed as a solubility enhancement formulation approach to brick dust molecules rather than to grease balls.

From the different types of nanotherapeutics, specific focus will be given to nanocrystals in the context of this review. Nanocrystals possess a high drug loading (close to 100%) in contrast to matrix nanoparticles consisting of polymeric or lipid matrices. Thus, the main advantage of nanocrystals is the low amount of excipients used allowing high drug concentration at the site of action and reduction of the potential toxicity of the excipients.

4. Advantages of nanocrystals in drug delivery

The increased saturation solubility and dissolution rate are the most important features of nanosuspensions. Regarding the saturation solubility, which for drug particles in the micrometre size range and above is a constant depending on temperature and dissolution medium, in the case of submicron particles, it depends on their size and is reported as 'apparent' saturation solubility [31]. The enhanced 'apparent' saturation solubility of nanosuspensions has been attributed to the increased curvature of nanoparticles resulting in increased dissolution pressure and hence drug solubility as described by a modified Kelvin and Ostwald-Freundlich equation (Eq.1)

$$ln\frac{S}{S_0} = \frac{2\gamma * V_m}{r * R * T} = \frac{2\gamma * M}{r * \rho * R * T} \quad . \quad . \quad . \quad \text{Equation 1}$$

where S is the drug solubility at temperature T, S_0 is the solubility of infinite big particle material, R is the gas constant, V_m is the molar volume, T is the temperature, r is the particle diameter, γ is the surface free energy, M and ρ are the molecular weight and density of the compound, respectively.

The reduced particle size and high surface area per unit mass of the nanoparticles lead to a more rapid dissolution as described by the Nernst and Brunner equation (Eq.2):

$$\frac{dm}{dt} = \frac{D * S}{h}(C_s - C) \quad . \quad . \quad . \quad \text{Equation 2}$$

where $\frac{dm}{dt}$ is the dissolution rate of non-formulated drug particles, *D* is the diffusion coefficient, *S* is the surface area of drug particles, *h* is the thickness of the diffusion layer, C_s is the saturation solubility of the drug particles and *m* is the concentration of the drug in solution. Therefore, by reducing the particle size, the total surface area, *S*, will increase resulting in a more rapid dissolution, particularly under sink conditions ($C << Cs$).

Moreover, according to the Prandtl equation (Eq. 3), the diffusion distance, *h*, is decreased for very small particles.

$$h_H = k \left(\frac{L^{1/2}}{V^{1/3}}\right). \quad . \quad . \text{ Equation 3}$$

where h_H is the hydrodynamic boundary layer thickness, k is a constant, V is the relative velocity of the flowing liquid against a flat surface and L is the length of the surface in the direction of the flow. Thus, apart from the surface effect, the simultaneous increase in the saturation solubility, C_s, and decrease in the diffusion distance, *h*, lead to an increase in the concentration gradient, $(C_s\text{-}C)/h$, thus increasing the dissolution rate according to the Nernst and Brunner equation (Eq. 2, [10]).

Due to the increased dissolution rate and enhanced saturation solubility, nanocrystals result in improved bioavailability [23,32]. Specifically, regarding oral drug delivery, nanocrystals have been used to address the issue of low bioavailability with reduced food effect compared to micronized drug [33]. Focusing on the influence of nanonization on the dissolution rate and saturation

solubility, the increase in dissolution rate remains the main effect of nanosizing while it is not clear to what extent the saturation solubility can be increased solely as function of smaller particle size [25]. Van Eerdenbrugh *et al.* [34] determined the solubility of crystalline drug nanosuspensions using various methods (e.g. separation-based methods, light scattering, turbidity). Based on the results of their study, solubility increases of only 15% were measured, highlighting that solubility increases due to nanosization are relatively small. These measurements are in agreement with what would be predicted based on the Ostwald-Freundlich equation (Equation 1). Solid state changes induced by particle breakage and increased surface wettability due to the presence of the stabilizer may also lead to enhancement of the 'apparent' saturation solubility and dissolution rate of nanosuspensions compared to micronized suspensions. Therefore, it is evident that the formulation and processing of drug nanocrystals are very important for their *in-vitro* and *in-vivo* performance.

Nanocrystals also enhance adhesiveness to the gastrointestinal mucosa, resulting in prolonged gastrointestinal residence and thus increased uptake via the gastrointestinal tract [35]. Jain *et al.* [36] incorporated nanosuspensions of ciprofloxacin into hydrogels; the formulations exhibited increased gastric residence time and satisfactory physical stability indicating their potential for the treatment of typhoid fever.

Formulating a drug as a nanosuspension has also been proposed as a method to mitigate challenges related to the chemical stability of solution formulations. For example, nanosuspensions of quercetin, a nutraceutical compound, appeared to be

photostable with no significant content loss over one month. In contrast, for the solution, a 28.3% reduction in drug content and discoloration were observed over the same period [37].

Apart from their superior clinical performance, nanosuspensions have attracted the interest of drug formulators as they can extend the life cycle of an active pharmaceutical ingredient (API) after patent expiration [23]. Moreover, nanosuspensions can be used as formulations during the whole drug development spectrum. Their quantitative and easy oral administration allows them to be used for preclinical animal studies [38], while due to the scalability of their production (e.g. wet milling), formulation amounts ranging from few mL up to a few liters can be generated. Small amounts are useful during preformulation stages while larger quantities are required during toxicological and pharmacokinetic studies in animals and for clinical trials under good manufacturing practices. All these characteristics have resulted in the rapid commercialization of nanosuspensions.

5. Stabilization of nanosuspensions

Nanosuspensions are thermodynamically unstable systems due to their large interfacial area, and thus they possess high interfacial free energy. The surface free energy (ΔG) termed 'Gibb's energy' associated with this area is given by Eq. 4:

$$\Delta G = \gamma_{SL} * \Delta A - T * \Delta S \quad . \quad . \quad . \quad \text{Equation 4}$$

where ΔA is the change in surface area, γ_{SL} is interfacial tension between the solid and liquid interface, T is the absolute temperature and ΔS is the change in entropy of the system. Therefore, the particles of a nanosuspension tend to aggregate in order to minimize the surface energy of the system.

For a nanosuspension to be stable it must contain a third component known as stabilizer additional to the solid particles and liquid, such as a surfactant and/or polymer. Kinetically, the process of aggregation depends on its activation energy. Addition of stabilizers suppresses aggregation by increasing the activation energy of the process [39].

The mechanisms of stabilization provided by the stabilizers can be classified as electrostatic repulsion and steric stabilization. Both mechanisms of stabilization can be achieved by incorporating ionic and non-ionic stabilizers into the nanosuspension medium. Stabilization by electrostatic repulsion can be explained by the DLVO theory [40].

Steric stabilization is mainly achieved by amphiphilic non-ionic stabilizers and can be described by the solvation effect. The non-ionic macromolecules orientate

themselves at the solid-liquid interface where they are adsorbed onto the particle surface through an anchor segment, while the well-solvated tail segment protrudes into the bulk medium. As two particles approach each other, the well-solvated segments of the stabilizer may interpenetrate. If the medium is a good solvent for the stabilizer molecules, the adsorbed segments on the particles cannot interpenetrate as the resultant desolvation is thermodynamically disfavoured [41]. Compared to electrostatic repulsion, steric stabilization is comparatively non-dependent on the presence of electrolytes in the medium and it is equally effective for both aqueous and non-aqueous dispersion media. Considering the changes of the pH along the gastrointestinal tract, steric stabilization exhibits advantages over electrostatic repulsion as a mechanism of stabilization.

Combination of the mechanisms of stabilization is often referred to as electrosteric stabilization. Electrosteric stabilization can be achieved by stabilizers which contain both a polymeric chain and charged groups (e.g. multi-amine containing polyelectrolytes, [42]) or by combining a non-ionic polymer and an ionic surfactant. Electrosteric stabilization has been suggested as a synergistic stabilization strategy due to the electrostatic repulsion between particles and enhanced steric hindrance from the adsorbed polymers [43].

Various types of generally recognised as safe (GRAS) pharmaceutical excipients have been used as stabilizers of drug nanosuspensions. Detailed reviews and tables on the use of polymers and surfactants as stabilizers of drug nanosuspensions are provided by Peltonen *et al.* [44] and Tuomela *et al.* [30].

The type and concentration of stabilizer used have been found to strongly influence the particle size and the size reduction kinetics of the nanosuspension produced [39,45]. Ito *et al.* [46] studied the effect of polymer species and concentration on the production of mefenamic acid nanosuspensions and they reported that there is a relationship between polymer affinity, solubilisation capacity and final particle size achieved. More specifically, they reported that there is an optimum stabilizer concentration for forming stable nanosuspensions with small particle size. When the stabilizer is present in the system in concentrations far above or below the optimum concentration, the nanosuspensions are prone to instability phenomena due to particle growth (Fig. 3). In the case of insufficient amount of stabilizer, the surface of the nanocrystals is not completely covered by the stabilizers and thus particle growth can be manifested due to particle aggregation. In the case of stabilizer overdosing, particle growth can be the result of Ostwald ripening in which larger particles grow at the expense of the smaller particles due to differences in solubility, as a function of the particle size [47].

Currently, the selection of a suitable stabilizer for a drug nanosuspension is based on trial and error. Few studies attempt to develop a rational approach for the selection of the appropriate stabilizer based on the physicochemical characteristics of the API in question. In this direction, George and Ghosh [48] studied the wet milling of six APIs with four different stabilizers to identify the material property variables (API and stabilizer) that control the critical quality parameters, which play a role in nanosuspension stability. They identified, the log *P*, the melting point and the enthalpy of fusion as the key drug properties that have a direct effect on nanosuspension stability. They highlighted that the most likely candidate for wet milling is a drug with a high enthalpy of fusion and hydrophobicity which can be

stabilized either electrostatically or sterically. At this point, it should be noted that other studies which investigated the stabilization of various drugs using different stabilizers at various concentrations have reported no correlation between the physicochemical characteristics of a drug (e.g. molecular weight, melting point, log P, solubility) and its feasibility to form a stable nanosuspension [49,50].

6. Formation of nanocrystals

Methods for the production of nanosuspensions can be categorised as top-down and bottom-up methods, depending on the starting material. In top-down methods, such as wet milling, high-pressure homogenization and microfluidisation, the starting material comprises larger solid particles than the resulting nanoparticles and mechanical processes are the fundamental mechanism causing particle size reduction. In bottom-up methods, particles are formed from the molecular level. Such methods are further subdivided into solvent evaporation (e.g. spray drying, electrospraying, cryogenic solvent evaporation, etc.) and antisolvent methods (e.g. liquid antisolvent, supercritical antisolvent, etc.) [51].

The main advantage of top-down over bottom-up methods is the production of nanosuspensions with high drug loading. Moreover, they do not involve harsh organic solvents since the solvent in which drug is dispersed, but not dissolved, is water for most poorly water-soluble drugs, making the top-down methods eco-friendly. This permits the formulation of many poorly soluble APIs, characterized as 'brick dust', suffering from poor solubility in a wide range of solvents. In general, because of the more streamlined process-flow and the solvent-free feature of top-down methods, most of the marketed and developmental nanosuspension-based pharmaceutical formulations have been produced by top-down methods.

From the various methods for the production of nanocrystals, the method of wet milling is considered in depth in the following section of this review, as it is the production method behind the majority of the marketed and developmental nanosuspension-based pharmaceutical formulations (Table 1).

7. Milling

Milling is a common physical unit operation for particle size reduction frequently applied in pharmaceutical formulation. During milling, mechanical energy imparts stress to particles which are strained and deformed. Fracture takes place through crack formation and crack propagation. For crystalline materials, fracture occurs preferentially along their crystal cleavage planes and increased concentration of crystal lattice imperfections makes fracture easier compared to crystals with fewer internal weaknesses. According to Heinicke [52], the main stress types applied in mills are compression, shear and impact, the latter can be further divided to stroke and collision. Wet milling will be discussed and its application in drug nanonization will be considered in more detail.

7.1. Wet milling

Milling a solid suspended in a liquid is referred to as wet milling. Experimental data on the wet milling of various materials suggest that the breakage rate kinetics (i.e. the median particle size versus milling time) follow a first-order exponential decay with longer milling times result in finer suspensions. The initial fast breakage of crystals can be attributed to the existence of more cracks and crystal defects in the larger crystals which propagate breakage relatively easy. After the initial fast breakage stage, size reduction continues but at a remarkably slower rate until a plateau is reached. The reduced rate of particle size reduction and finally the achievement of a plateau (steady state) suggest that in the later stages of wet milling the mechanism of fracture changes. As the particle size decreases with increasing milling time, the shear stress of the suspension increases and thus attrition becomes the dominant mechanism of comminution [53].

Understanding of the breakage kinetics for a specific drug and milling set up is important for determining the milling duration that should be selected in order to achieve particles of the desired fineness. Various mathematical modelling approaches have been developed to describe the impact of process parameters (e.g. milling speed, bead concentration, drug loading, etc.) on the breakage kinetics and particle size distribution. These modelling approaches extend from purely descriptive dynamic models to discrete element modelling, population balance models and microhydrodynamic models. A detailed review on the models that have been developed for enhanced understanding of milling processes is provided by Bilgili *et al.* [54].

Regarding pharmaceutical manufacturing, the two most common types of wet mills used are: the rotor-stator and the media mills.

7.2. Rotor-stator mixers/wet mills

Rotor-stator mixers consist of a high-speed mixing element (the rotor) in close proximity with a static element (the stator). They are also referred to as high-shear devices as the shear rates generated in these devices are orders of magnitude higher than in a conventional mechanically stirred vessel. Rotor-stator mixers are mainly used for homogenization and emulsification purposes. However, the common action of the rotor and the stator results in shear stress, turbulence and cavitation forces which apart from mixing lead to size reduction [55].

7.3. Wet media mills

The second type of mills used for wet milling are media mills. Wet media milling involves feeding the milling chamber with the milling media (e.g. milling beads), the particulate material, the stabilizer and a suitable solvent or mixture of solvents.

The milling beads are made of a hard and dense material such as yttrium-stabilized zirconium oxide, stainless steel, glass alumina, titanium or certain polymers as highly cross-linked polystyrene and methacrylate. The beads size may vary from less than 0.1 mm to 20 mm. As a rule of thumb, the smaller the size of the milling beads the finer the nanoparticles produced, due to increased collision frequency between drug particles and beads. However, too small beads (e.g. 0.03 mm) may not be suitable for milling as they cannot generate sufficient energy for particle breakage when they impact with drug particles due to their light weight.

7.3.1. Wet media milling equipment

Wet media milling equipment that used for the production of nanosuspension can be divided into planetary ball mills and wet stirred media mills. Planetary ball mills are high-energy ball mills and their name is derived from the kinematics of the grinding components which are analogous to the rotation of the earth around the sun. A planetary mill is usually made of two or more jars, rotating at an angular velocity (ω) around their axis, installed on a disk rotating at an angular velocity (Ω, Fig. 4). Usually for colloidal milling, the ratio between the speed of the rotating disk and the milling jar is 1: -2, this means that during one rotation of the disk the jar rotates twice in the opposite direction. Comminution occurs by impact, frictional and shear forces resulting from collision among the particles, the milling media and the wall of the milling jars. Coriolis and centrifugal forces lead to a rapid

acceleration of the milling media which results in the production of particles in the submicron range [56].

Apart from some newly launched models (e.g. E_{max}, Retsch), the majority of planetary ball mills do not have any integrated cooling system. This means that a major part of the energy introduced into the milling chamber is transformed into heat and dissipated into the suspension. The increase of temperature during milling is considered as an additional mechanism behind the reduction of particle size. Steiner *et al.* [57] prepared nanosuspensions of lactose in ethanol and reported a strong influence of the suspension temperature on the resulting particle fineness.

Planetary ball mills are mainly used for the development of drug nanosuspensions at the laboratory scale due to their mechanical simplicity and versatility. Wet milling using planetary ball mills has been successfully employed to produce nanosuspensions for drugs such as indometacin and brinzolamide [45,58]. Based on the principle of planetary ball milling, Juhnke *et al.* [59] developed a screening media milling equipped with up to 24 milling beakers of 0.05-1.0 mL individual milling chamber volume. Scaling-up studies to a laboratory stirred media mill resulted in satisfactory comparability, indicating that a particle formulation optimised in a planetary ball mill can be transferred to other mill types which are used for the production of larger batch size. Therefore, the screening media mill is a useful tool for the accelerated preclinical and clinical pharmaceutical development of formulations based on nanomilling.

Wet stirred media mills are the most commonly used type of mills to produce drug nanosuspensions. In stirred media mills, milling media are moved by a rotating agitator and production of submicron particles can be achieved due to a very high

number of stress events per unit time and unit volume and due to an appropriate stress intensity [60]. The mills used to produce nanosuspensions are high-speed, closed-type stirred media mills, operating at circumferential stirrer speed of 8 to 20 m s^{-1}. They are equipped with a separation device (screen or rotating gap) which allows the free discharge of the product but prevents the milling media from leaving the chamber. Mill designs vary in the chamber volume capacity (ranging from less than 1 L to more than 1 m^3) and the stirrer geometry (e.g. disk, pin-counter-pin stirrer). Usually, this type of mills is equipped with a cooling system allowing precise temperature control but also processing of thermolabile compounds as product overheating can be prevented. Detailed studies on the impact of process parameters on the breakage kinetics of poorly water-soluble drugs have been provided by Afolabi *et al.* [61] and Li *et al.* [62].

Wet stirred media mills can operate in batch, recirculation or continuous mode. Batch mode is mainly restricted in the development of nanosuspensions at the laboratory scale. In recirculation mode, a recirculation pump and a holding tank are added in the milling set-up (Fig. 5). The pump is employed to circulate the suspension from the holding tank, through the mill, and back into the holding tank, allowing the production of a fixed batch size as determined by the capacity of the holding tank [63]. In continuous operation, a receiving tank is also used allowing the continuous withdrawal of product from the mill. There are two types of continuous mode: the multi-pass continuous and the cascade-continuous mode. In multi-pass continuous mode, the suspension flows from the holding tank, through the mill and into the receiving tank while in the cascade-continuous mode, the suspension flows from the holding tank, through mills in series and into the receiving tank [63]. The fact that wet stirred media milling can be employed in a

continuous mode is a significant advantage of the process as nowadays the pharmaceutical manufacturing sector is moving towards the implementation of continuous processing strategies.

8. Applications of nanocrystals in drug delivery

8.1. Oral drug delivery

Oral drug delivery is the most popular and convenient route of administration for nanocrystalline-based products. As presented in Table 1, these products have been developed either as liquid oral dosage forms (i.e. suspensions) or as solid oral dosage forms (i.e. tablets and capsules). Regarding the solid oral dosage forms, a solidification step is employed after the preparation of nanosuspensions. Spray and freeze drying (lyophilization) are the most commonly used techniques while fluidised-bed coating, granulation and pelletisation yield formulations with more straightforward downstream processing to tablets or capsules. Other techniques such as spray-freeze drying, aerosol flow reactor and printing, which are less frequently applied in pharmaceutical technology have also been employed [64]. It is important for the solid nanocrystalline-based formulations to retain their redispersibility (i.e. ability to reform nanoparticles upon rehydration) as it is a prerequisite for their superior clinical performance. For this purpose, addition of matrix formers (e.g. sugars) is a common strategy in order to produce redispersible solid nanocrystalline formulations [65].

Rapamune® (Wyeth) is a nanocrystalline-based formulation of the macrocyclic immunosuppressive drug sirolimus (rapamycin). It was the first nanocrystalline product to reach the market and is available in two formulations: oral suspension and tablets [10]. The product was developed using Elan's NanoCrystal® technology in order to eliminate limitations related to the first commercially available formulation of sirolimus, which is a viscous oral solution of the drug in Phosal 50 PG and polysorbate 80. The lipid-based liquid solution needs to be

refrigerated and protected from light upon storage, it is unpalatable and its dispensing protocol is complicated [66]. Rapamune® tablets, on the other hand, exhibited a 27% increase in the bioavailability of the drug compared to the lipid-based solution and their ease of administration contributes to enhanced patient adherence to medication [67].

Emend® (Merck) is a nanocrystalline-based product of the antiemetic drug aprepitant which was developed using Elan's NanoCrystal® technology. It is formulated as capsules containing sugar beads coated with an aprepitant nanosuspension. Nanonisation of aprepitant eliminated the high fasted/fed state variation related to the conventional micronized formulation used in early clinical studies [33]. A similar concept can be found behind the development of Megace ES® (Par Pharmaceutical) which is a ready-to-use liquid nanosuspension of megestrol acetate. Megace ES® is indicated as an appetite stimulant for the treatment of anorexia and weight loss in patients with HIV. While the oral solution of megestrol acetate exhibited significant food effect, Megace ES® managed to increase the bioavailability of the drug and reduce the food effect, thus allowing the administration of the drug in the fasted state. Considering that the patient population for this drug exhibits difficulties in consuming food, Megace ES® as a stable and non-viscous nanosuspension contributed to enhanced patient adherence to medication [67].

Tricor® (Abbott) is a nanocrystalline-based formulation of fenofibrate for the treatment of hypercholesterolemia. The product is based on Elan's NanoCrystal® technology and it is available in the form of tablets. Launching Tricor® as a

nanocrystalline formulation was part of the company's strategy involving the sequential launch of branded reformulations of fenofibrate in order to maintain a dominant market share years after generic competition was permitted [68].

8.2. Parenteral drug delivery

Via the parenteral route of administration (i.e. subcutaneous, intramuscular, intravenous, intradermal and intra-arterial injection) the drug can be administered directly into a blood vessel, organ, tissue or lesion. Nanotherapeutics hold great potential for selective and controlled delivery of drugs to target cells and organs [69,70]. Two additional advantages of nanocrystals regarding parenteral drug delivery are the high drug loading and the ease of sterilisation of these formulations using conventional methods including gamma irradiation, filtration and thermal sterilisation [71]. Currently, several poorly water-soluble drugs have been formulated as nanocrystals for intravenous, intramuscular and intraperitoneal administration [72]. At this point, it should be highlighted that for nanosuspensions intended to be administered intravenously, the particle size stability of nanocrystals upon storage is of paramount importance and the content of particles larger than 5 μm should be controlled strictly to avoid capillary blockade and embolism.

Regarding intravenous (IV) administration, a few studies have reported the development of nanocrystals as tumour-targeting drug delivery approach. The main impetus to formulate drugs as nanocrystals for IV administration has been the enhanced permeation and retention effect that facilitates passive accumulation of particles (20-300 nm) in tumour tissues. Shegokar *et al.* [73] prepared nanosuspensions of the antiretroviral drug nevirapine (457 ± 10 nm) for HIV/AIDS chemotherapy. The nanosuspensions were further surface-modified by stabilizer adsorption, e.g. serum albumin, polysaccharide and PEG 1000. The non-modified and surface-modified nanosuspensions were tested for their targeting potential to the mononuclear phagocytic system cells by *in-vitro* protein adsorption studies

using two-dimensional polyacrylamide gel electrophoresis. In the adsorption patterns of both non-modified and surface-modified nanosuspensions, high amounts of immunoglobulins were determined indicating uptake by the liver and spleen. In a follow-up study, the biodistribution, uptake and toxicity profiles of the nanosuspensions (non-modified and surface-modified) were tested after IV administration to rats and compared to the plain drug solution. Surface-modified nanosuspensions exhibited improved drug accumulation in various organs of the rat such as the brain, liver and spleen, suggesting that nanonisation of nevirapine significantly improved its *in-vivo* behaviour and thus is a promising formulation approach for targeting antiretroviral drugs for HIV/AIDS to cellular reservoirs [73].

InvegaSustenna® (Johnson & Johnson) is an extended-release nanosuspension of the antipsychotic drug paliperidone palmitate which has been found effective in controlling the acute symptoms of schizophrenia and delaying relapse of the disease. The formulation, as a nanosuspension, was developed using Elan's NanoCrystal® technology. The product is available in ready-to-use prefilled syringes and is administered once-monthly by intramuscular injection following a specific protocol which consists of an initial dosing and a maintenance dosing period. The concept behind the development of Invega Sustenna® is different compared to the other nanocrystalline-based products. In other words, paliperidone (parent drug) does not exhibit any solubility issues and its conversion to paliperidone palmitate (prodrug) in combination with its nanonization is an approach for limiting its solubility and thus sustaining drug release [67]. That InvegaSustenna® is administered once-monthly is a great advantage giving increased product safety, tolerability and most importantly improved patient

adherence to medication compared to other antipsychotic drugs that require daily dosing.

8.3. Pulmonary drug delivery

Many of the advantages outlined in section 4 can be extended to pulmonary drug delivery. Regarding drug delivery to the lungs, drug absorption and local bioavailability depend upon the fraction of the drug which is deposited and dissolved in the lung fluids. Once the particle has deposited on the lung surface, mucociliary clearance and drug absorption are two competitive mechanisms influencing the fate of the drug. Specifically, when mucociliary clearance takes place faster than drug absorption, as in the case of drugs with low dissolution rate, this can lead to reduction in the bioavailability. Formulations consisting of nanoparticles have been found to promote more rapid absorption following inhalation of poorly water-soluble drugs which suffer from dissolution-limited absorption (e.g. beclometasone dipropionate, budesonide, itraconazole, [74]). Nanosuspensions have been proposed as a formulation approach to increase the dissolution rate and thus the absorption of poorly water-soluble inhaled corticosteroids such as fluticasone propionate and budesonide which constitute indispensable drugs in the armamentarium against asthma and other respiratory diseases [75]. Britland *et al.* [76] compared the bioavailability, emission characteristics and efficacy of a budesonide nanosuspension with those of a micronized suspension of the drug after delivery as a nebulised aerosol to a human airway epithelial culture cell line. For an equivalent dose, the budesonide nanosuspension achieved improved uptake, retention and efficacy in the culture cells.

Apart from the use of nanosuspensions in nebulisers, solidification of nanosuspensions to respirable nanoparticle agglomerates (aerodynamic diameter between 1-5 μm) has been applied to prepare dry powders for inhalation [77,78]. According to El-Gendy *et al.* [79,80], the controlled agglomeration of nanosuspensions to inhalable nanoparticle agglomerates is *"an approach to harmonise the advantages of nanoparticles with the aerodynamics of small microparticles so as to achieve an improved bioavailability and aerosolization behaviour of the drug"*. Production of nanosuspension by wet media milling and subsequent solidification by spray drying after the addition of GRAS excipients, such as mannitol (matrix former) and L-leucine (aerosolization enhancer), has been applied as a platform for the formation of respirable nanoparticle agglomerates. The nanoparticle agglomerates produced by this platform were found to exhibit enhanced aerosolization and dissolution performance while they retained their crystallinity, which is beneficial for their long-term stability upon storage [81]. By careful selection of the formulation and process parameters, which can be facilitated using design of experiments methodology, this platform can be successfully applied to various drugs with different physicochemical properties (Fig. 6, [78,82]).

8.4. Ocular drug delivery

Ocular drug delivery is the preferred route of administration for pathologies of the eye such as infections, inflammation, dry eye syndrome, glaucoma and retinopathies. The complex structure and nature of the eye poses challenges to formulation scientists due to the very low ocular drug bioavailabilty (usually less than 5%). Research has focused on nanocarrier-based drug delivery systems (e.g. liposomes, polymeric micelles) as they are capable of overcoming many of the biological barriers of the eye and thus enhancing ocular drug bioavailability. Recently, the use of nanocrystals as an ocular formulation approach for poorly water-soluble drugs is gaining popularity and this can be attributed to the faster clinical development and commercialisation of nanocrystals compared to other types of nanotherapeutics such as liposomes and dendrimers [83]. According to Sharma *et al.* [84], the advantages of nanocrystals for drug delivery to the eye are: improved ocular safety, increased retention of the formulation in cul-de-sac, enhanced corneal permeability across the corneal and conjunctival epithelium, enhanced ocular bioavailability, dual drug release profile in the eye and increased tolerability. Specifically, the dual drug release profile of nanocrystals in the eye means that they exhibit both immediate and sustained drug release profiles after their topical administration. The immediate drug release can be linked to the increased saturation solubility and dissolution of the nanocrystals resulting in initial higher concentrations available for absorption and thus rapid onset of action. The prolonged drug release, on the other hand, derives from the high surface area of the nanocrystals which facilitates interactions with biological membranes. The increased interactions with the ocular mucosa provide nanocrystals with mucoadhesive properties, increasing their retention time in the cul-de-sac region

and thus prolonged drug action is achieved. Increasing the viscosity of nanosuspensions or inclusion of nanocrystals into an in-situ gelling system can further increase the retention time and thus prolong the release profile of the drug [85].

Tuomela et al. [58] prepared nanosuspensions of the poorly water-soluble drug brinzolamide as ocular formulations for the treatment of glaucoma. From the polymers/surfactants that were screened as stabilizers during wet media milling, hydroxypropyl methylcellulose was found to be the stabilizer of choice as it was capable of maintaining the reduced particle size of the nanosuspensions (~ 460 nm). Both the cell viability results and the intraocular pressure effect achieved with the nanosuspensions were comparable with the marketed formulation of the drug (Azopt®: eye drops containing nanocrystals of brinzolamide stabilized with tyloxapol).

8.5. Dermal drug delivery

Dermal delivery of nanocrystals was a route of administration that was not fully exploited until lately, despite the advantages of nanocrystals such as adhesion, fast dissolution and increased penetration that can be of great importance for dermal application. The development of nanocrystals for delivery to the skin was firstly exploited in the field of cosmetics and it was later expanded for drug delivery purposes [23]. Specifically, the cosmetic products Juvedical ® (Juvena of Switzerland, Juvena Marlies Möller AG) and Platinum Rare collection (La Prairie ®) contain nanocrystals of the antioxidants rutin and hesperidin, respectively. Incorporation of nanocrystals into these cosmetic products is straightforward as the aqueous nanosuspension is mixed with the cosmetic product (e.g. cream, lotion).

Currently, apart from a wide range of antioxidants, drugs such as caffeine and diclofenac acid have been formulated as nanosuspensions for dermal application [86,87]. According to Vidlárova *et al.* [88], optimal dermal nanocrystal formulations should combine the following features: increased concentration gradient due to higher kinetic saturation solubility, low density of nanocrystals on the skin surface to cover densely enough the skin and sufficiently large area of direct contact of crystal surface to lipid films of the stratum corneum.

Lai *et al.* [89] prepared nanosuspensions and nanoemulsions (oil-in-water) of tretinoin, an active compound widely used for the treatment of acne vulgaris. Dermal and transdermal delivery of both tretinoin nanoformulations were tested *in vitro* using Franz cells and newborn pig skin. Formulating tretinoin as a

nanosupension was found to favour drug accumulation into the skin (dermal delivery) and to minimize diffusion of the drug through the skin into the systemic circulation (transdermal delivery). On the contrary, a nanoemulsion is useful to improve both dermal and transdermal delivery. Moreover, photodegradation studies, using UV irradiation of the formulations, revealed that the nanosuspension could improve tretinoin's photostability compared to the nanoemulsion and the methanolic solution of the drug. Therefore, formulating tretinoin as nanosuspension appears to be a useful formulation approach for improving both the dermal delivery and stability of the drug.

9. Concluding remarks

The number of drug candidates suffering from poor aqueous solubility is on the rise making poor solubility a major challenge for the pharmaceutical industry. Nanocrystals are nanosized drug particles produced as nanosuspensions in the presence of a stabilizer in order to achieve colloidal stability. Nanocrystals combine the advantages of increased saturation solubility and faster dissolution rate leading to enhanced bioavailability and reduced food effect for many drugs. Chemical stability and low toxicity of nanocrystals due to their high drug loading are also beneficial aspects of this formulation approach.

Various methods have been investigated and patented for the preparation of nanosuspensions that can be classified as top-down (e.g. wet milling, high pressure homogenisation) and bottom-up techniques (e.g. antisolvent precipitation). Milling has a long history as a unit operation in pharmaceutical technology, but it is the advent of new devices with increased rotational speed and finer milling media that allows the use of milling as a nanonization technique. Currently, wet milling is the method behind most of the marketed nanocrystalline-based products. Planetary ball mills and wet stirred media mills are the main types of equipment that have been used to produce nanosuspensions, the first mainly for laboratory-scale production and the latter for scaling-up purposes. The variety of poorly water-soluble drugs that have been processed to nanosuspensions using wet milling indicates the universality and versatility of this nanonization technique. Careful selection and optimisation of process and formulation parameters can extend the use of wet milling to almost any drug.

Nanocrystalline-based formulations either as liquid nanosuspensions or after downstream processing to solid dosage forms have been mainly developed as oral and parenteral drug delivery systems. However, nanocrystalline-based formulations have been found to exhibit unique advantages for targeted delivery to the lungs, the eye and the skin. In conclusion, the number of nanocrystalline-based products already commercially available together with the increasing number of scientific research and patents on drug nanocrystals for various applications indicate that both pharmaceutical industry and academia have embraced this universal formulation approach, which is expected to advance even more in the near future.

Conflicts of interest

The authors declare no conflicts of interest.

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FIGURES

Figure 1. A nanoscale comparison and types of nanotherapeutics used in drug delivery.

Figure 2. Scanning electron microscopy images (top) of (a) the poorly water-soluble antifungal drug, posaconazole starting material and (b) posaconazole nanocrystals produced by wet milling. The particle size distribution graphs (bottom) were determined using laser light diffraction after suitable dilution by distilled water. Reproduced with permission from [15].

Figure 3. A suitable concentration of stabilizer should be present in the system to produce nanosuspensions with small particle size and to assure colloidal stability. Excess stabilizer should be avoided to prevent solubilisation and increase of particle size due to Ostwald ripening. Adapted with permission from [46].

Figure 4. Schematic drawing of a planetary ball mill: (a) three-dimensional view, (b) top and (c) sectional view. R_j: the jar radius, R_p: the disk radius, ω: angular velocity of grinding jar around the planetary axis and Ω: angular velocity of rotating disk around the sun axis. Reproduced with permission from [56].

Figure 5. Schematic drawing of a wet stirred media mill (Microcer model, Netzsch Fine Particle Technology, USA) operating in the recirculation mode. P and T stand for pressure and temperature sensor, respectively. Reproduced with permission from [61].

Figure 6. Preparation of respirable nanoparticle agglomerates by combining wet milling and spray drying. “Road map” developed to guide the selection of formulation and process parameters that should be adjusted to engineer inhalable nanoparticle agglomerates, by considering the physicochemical properties of the drug in question.

FIGURES

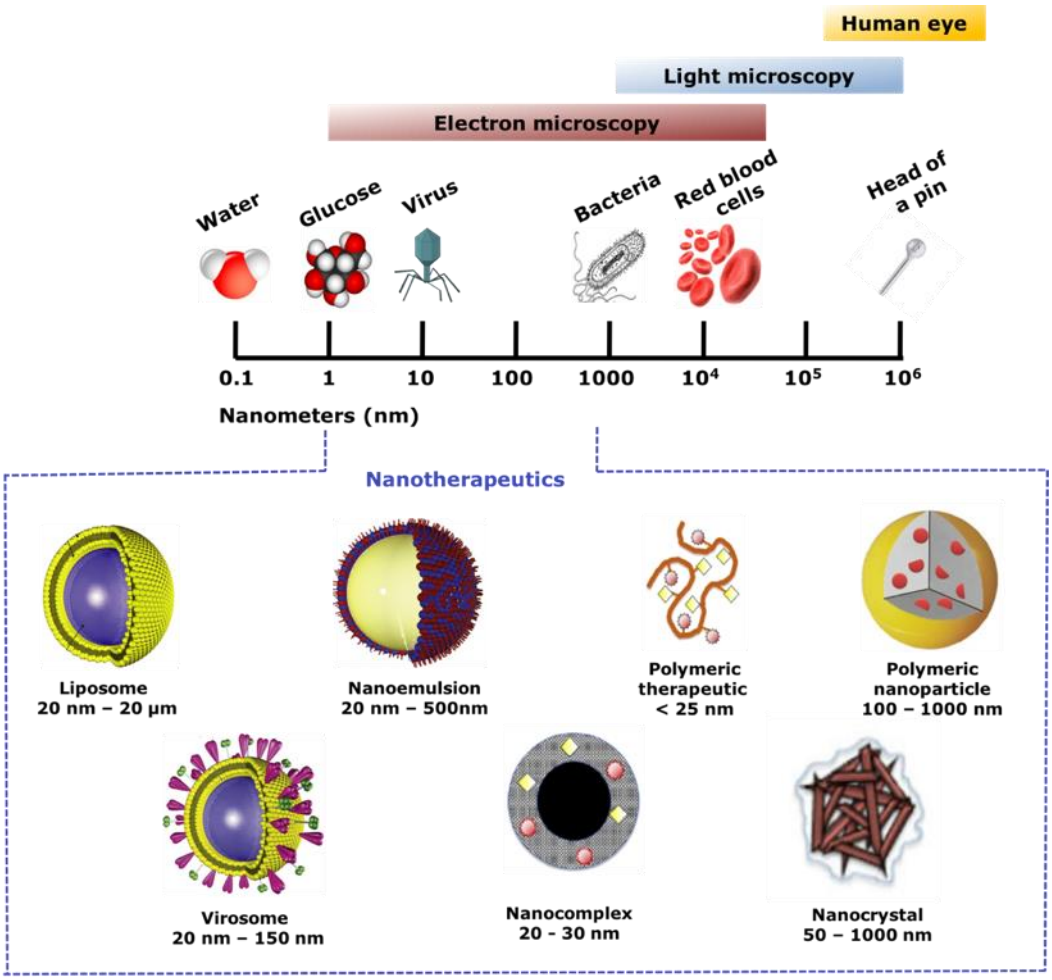

Figure 1

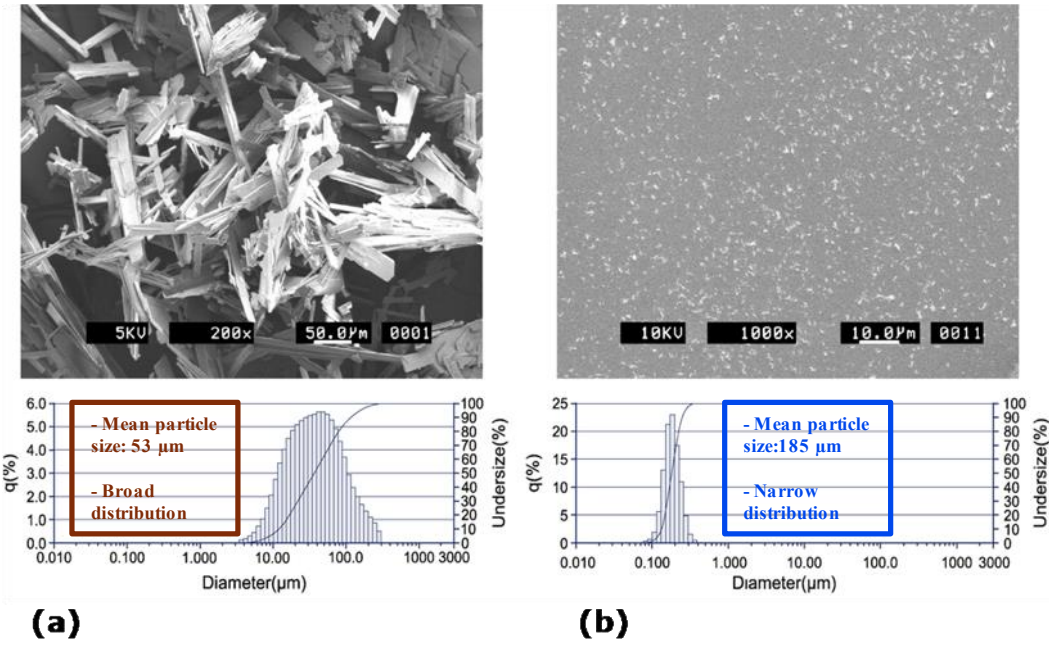
5KV 200x 50.0µm 0001
10KV 1000x 10.0µm 0011
- Mean particle size: 53 µm
- Broad distribution
- Mean particle size:185 µm
- Narrow distribution
q(%)
Undersize(%)
Diameter(µm)
0.010 0.100 1.000 10.00 100.0 1000 3000
(a)
(b)

Figure 2

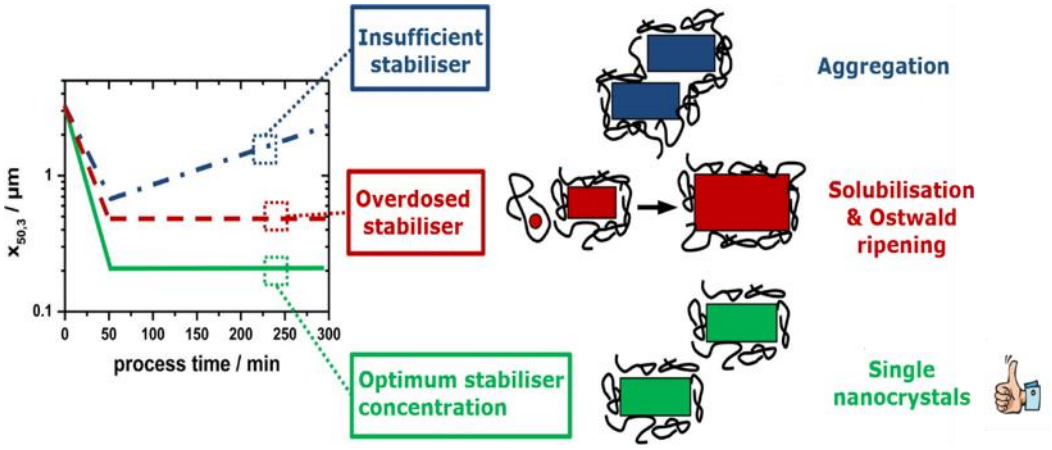
Insufficient stabiliser
Overdosed stabiliser
Optimum stabiliser concentration
$x_{50,3}$ / µm
1
0.1
0
50
100
150
200
250
300
process time / min
Aggregation
Solubilisation & Ostwald ripening
Single nanocrystals

Figure 3

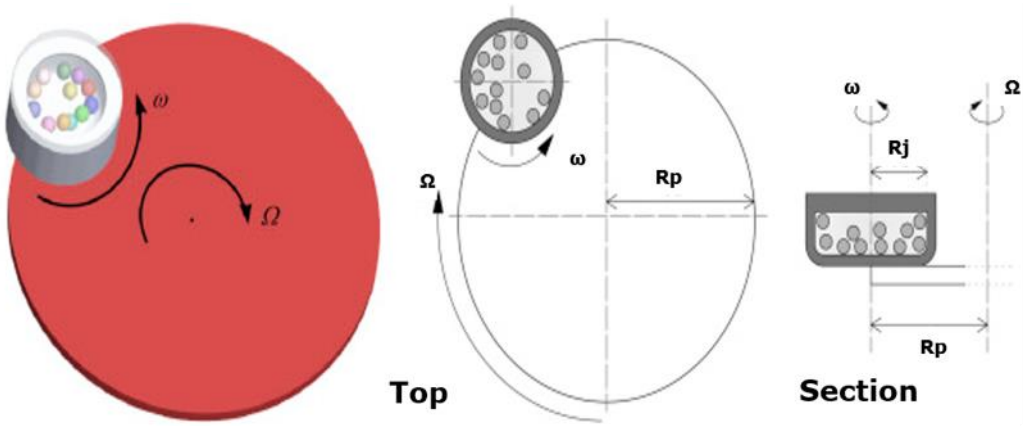

Figure 4

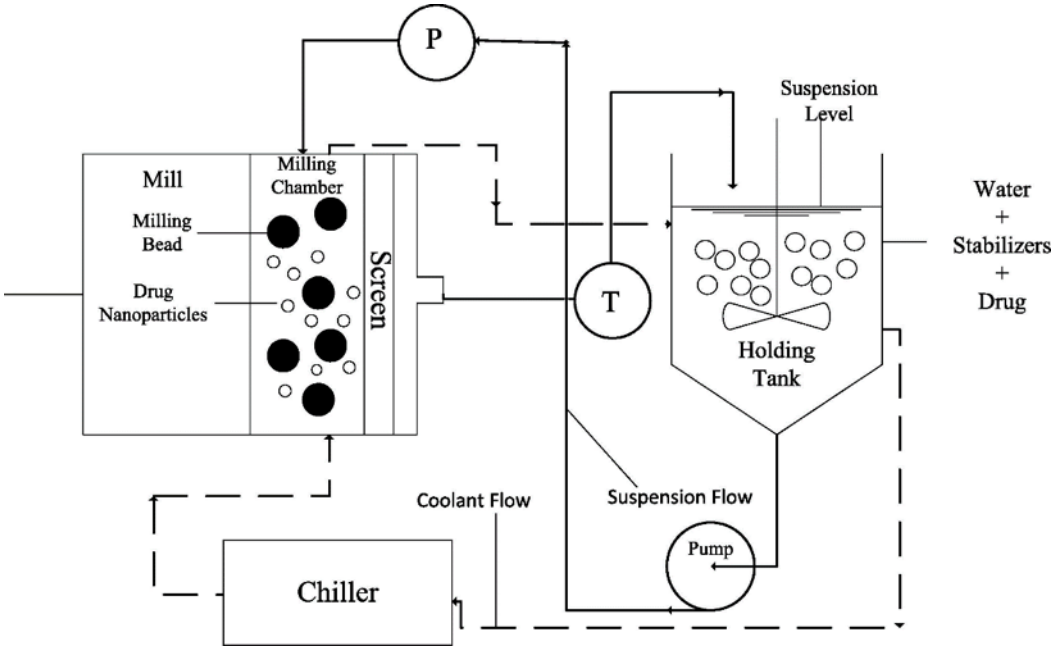
P
Suspension
Level
Mill
Milling
Chamber
Milling
Bead
Drug
Nanoparticles
Screen
T
Water
+
Stabilizers
+
Drug
Holding
Tank
Coolant Flow
Suspension Flow
Pump
Chiller

Figure 5

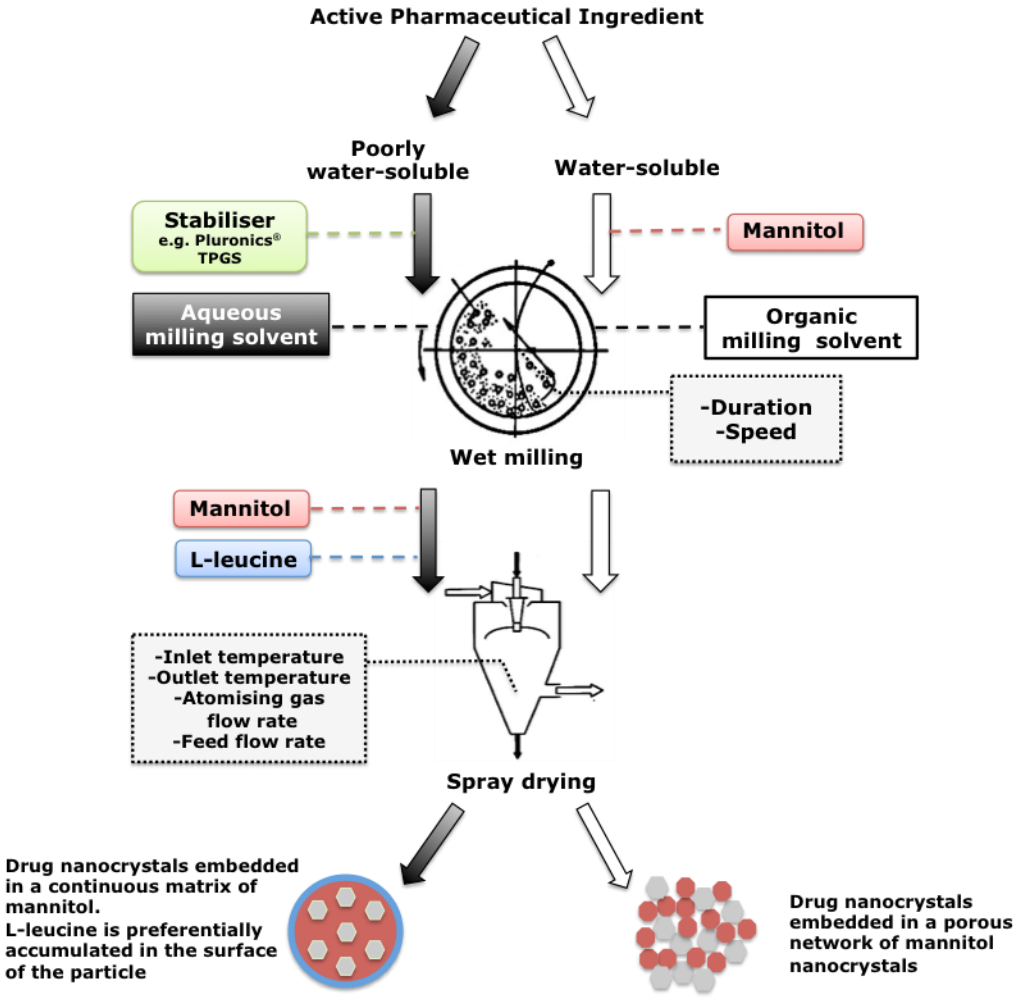
Active Pharmaceutical Ingredient
Poorly water-soluble
Water-soluble
Stabiliser
e.g. Pluronics®
TPGS
Mannitol
Aqueous milling solvent
Organic milling solvent
-Duration
-Speed
Wet milling
Mannitol
L-leucine
-Inlet temperature
-Outlet temperature
-Atomising gas flow rate
-Feed flow rate
Spray drying
Drug nanocrystals embedded in a continuous matrix of mannitol.
L-leucine is preferentially accumulated in the surface of the particle
Drug nanocrystals embedded in a porous network of mannitol nanocrystals

Figure 6