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Review

# Nanocrystals of poorly soluble drugs: drug bioavailability and physicochemical stability

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**Abstract:** Many approaches have been developed over time to counter the bioavailability limitations of poorly soluble drugs. With advances in nanotechnology in recent decades, science and industry have been approaching this issue through the formulation of drugs as nanocrystals, which consist of pure drugs and a minimum of surface active agents required for stabilization. They are carrier-free submicron colloidal drug delivery systems with a mean particle size in the nanometer range, typically between 10 and 800 nm. By reducing particle size to nanoscale, the particle surface area available for the molecule dissolution in the direction of dissolution medium is increased, and thus bioavailability is enhanced. This approach has proven successful, as demonstrated by the number of such drug products on the market. R&D and industry have offered many technological solutions to reduce the particle size to nanoscale, and also devised solutions for the handling of particle of nanodimensions, such as methods to accurately measure nanoparticle size and techniques to prevent physicochemical and stability related problems, such as aggregation. The present work provides an overview of the more recent achievements in improving the bioavailability of poorly soluble drugs according to their administration route, and describes the methods developed to overcome physicochemical and stability related problems.

**Keywords:** Nanocrystals; poorly soluble drug; nanotechnology; stability

## 1. INTRODUCTION

It is estimated that 90% of new drugs in the development pipeline can be classified as poorly soluble [1]. Given the great number of poorly soluble drugs, innovative and appropriate formulations as well as technological solutions are needed to sufficiently increase drug bioavailability, accordingly to the administration route [2].

To date, the classical approach for increasing the dissolution rate of poorly soluble drugs is to reduce particle size, in particular through micronization [3]. However, it seems that further improvement in drug dissolution rate and thus in bioavailability demands a move from micronization to nanonization. This requires different and innovative technological approaches, as well as innovative solutions to overcome all the physicochemical and stability problems associated with nanostructures.

Thanks to the advanced process technologies and analytical methods developed in last decades, a considerable number of pharmaceutical nanocrystal products are now on the market and several are under development [4]. Nanocrystals, consisting of pure drugs and a minimum of surface active agents required for stabilization, are carrier-free submicron colloidal drug delivery systems with a mean particle size in the nanometer range, typically between 10 and 800 nm [5]. Nanocrystals offer several advantages and disadvantages, which are described in depth in Table 1.

Table 1: Description of the main advantages and disadvantages of nanocrystals.

| ADVANTAGES OF NANOCRYSTALS | REFERENCES |
|---|---|
| Different administration routes (oral, intravenous, intramuscular, pulmonary, ocular, dermal) | [6] [7-11] [12, 13] [14-26] [22] |
| Different pharmaceutical dosage forms | [6] [27-31] |
| Higher saturation solubility than conventional particles | [6] [20] [24] [26] [32-34] |
| Faster dissolution rate than conventional particles | [3] [7, 8] [11] [33] [35] [24] [36, 37] |
| Potential for passive and active targeting of drugs | [38, 39] |
| Long circulating nanocrystals | [40-42] |
| Reduced tissue irritation in case of subcutaneous/intramuscular administration | [43] |
| Hybrid nanocrystals | [16, 44-46] |
| DISADVANTAGES OF NANOCRYSTALS | REFERENCES |
| Physicochemical related stability problems | [4] [36] [47-49] |
| Bulking sufficient care must be taken during handling and transport | [50, 51] |
| Uniform and accurate dose cannot be achieved | [52, 53] |

A brief summary of terminology is useful. First, the term nanocrystal has a different meaning than the term nanoparticle. "Nanocrystal" is often used undiscriminatingly to refer to the drug solid state, specifically for solid particles in the nanometric range with minimum excipients. "Nanoparticle" refers more generally to particles in the nanometric range dimension mainly composed of polymers or lipids, for example polymeric nanoparticles, liposomes, solid lipid nanoparticles, etc. Thus, in the case of nanocrystals, the term "nanoparticles" is not preferred because it is ambiguous. It is true that in several cases, these "nanocrystals" exist in an amorphous physical state, but this should not justify the use of the term "crystal". Some have avoided the ambiguity by using the term "pure solid nanoparticles" to refer to solid pure nanoparticles, without any reference to the drug solid physical state [54]. When referring to formulations where nanoparticles are dispersed in a liquid, the preferred term is "nanosuspension".

In the present review, the term "nanocrystal" will be generally preferred in reference to the solid physical state, because it is generally used, accepted, and understood by the scientific community.

This review has two objectives:

a. to overview the more recent information about the improvement in dissolution rate and bioavailability of poorly soluble drugs, formulated as nanocrystals and administered through different administration routes;
b. to review the physicochemical stability related problems of nanocrystals and the methodological approaches to improve the physicochemical stability of formulations.

The focus is on scientific works of the last decade, with a few exceptions.

## 2. PRODUCTION TECHNOLOGIES OF NANOCRYSTALS

Briefly, nanoparticles can be produced through top-down and bottom-up technologies. Top-down approaches involve the reduction of large particles to the nanometer range, for example, by milling, while bottom-up methods generate nanoparticles by building them from drug molecules in solution, such as by precipitation. Some approaches can apply in succession two technologies, defined as combined technologies. Table 2 provides a general overview of these methods.

For a detailed examination of the methods for the preparation and production of nanocrystals, the reader is encouraged to consult the following publications [32, 55-61].

74 **T**able 2: Main technologies applied for the production of nanocrystals (nanosuspensions). References
75 were approximately focused on the last ten years.

| TECHNIQUE | | | DRUG | REFERENCES |
|---|---|---|---|---|
| Bottom-Up | Evaporation methods | Spray drying | Budesonide Nicergolide, Indomethacin | [18] [37] [54] [62] |
| | | Freeze drying | Fenofibrate | [9] |
| | | Areosol flow reactor | Beclomethasone dipropionate | [63] |
| | | Electrospraying | Insulin | [64] |
| | Precipitation methods | Solvent-antisolvent precipitation | Paclitaxel | [65] |
| | | High gravity precipitation | Cefuroxime axetil, Cephradine, Azithromycin, Danazol, Salbutamol sulphate | [66-69] |
| | | Flash precipitation | Cyclosporine A | [70] |
| | | Sonoprecipitation | Fenofibrate, Paclitaxel | [9] [65] |
| | | Supercritical fluid | Apigenin | [8] |
| Top-Down | High pressure homogenizatio n | Microfluidification | Bexarotene | [10] |
| | | Piston gap homogenization | Nimodipine, Lutein, Asulacrine, Baicalein, Apigenin, Quercetine, Hesperitin, Resveratrol, Indomethacin, Hydrocortisone acetate, Nevirapine, Amphotericin B | [6] [11] [13] [15] [19] [28] [71-75] |

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| | | | | |
|---|---|---|---|---|
| | Bead milling | | Apigenin, Dexamethasone, Ibuprofen, Tacrolimus, Quercetine | [24] [71] [76] |
| | Cryo-milling | | Indomethacin (IDM), Glibenclamide, Ketoconazole, Ursodiol, Indomethacin, Griseofulvin, Carbamazepine, Piroxicam | [49] [62] [77] |

## 3. DRUG BIOAVAILABILITY OF NANO-FORMULATIONS

The bioavailability of a drug depends on its ability to dissolve in biological fluids, to cross membranes, and to efficiently reach its pharmacological target. In the biopharmaceutical classification of drugs [78], drugs of Class II group are characterized by poor solubility, but have a good ability to cross membranes. Thus, to improve the bioavailability of a Class II drug, it is necessary to increase drug solubility and/or drug dissolution rate.

In particular, for nanocrystals, it is possible to consider the following scenarios:

1. A decrease in particle size leads to an increase in surface area available for the interaction with the dissolution media and thus an increase in particle dissolution rate, in accordance with the Noyes-Whitney law [10, 79, 80].
2. An increase in the particle curvature (particularly pronounced for colloidal particles) leads to an increase in dissolution pressure, according to the Kelvin's equation [81].
3. When kinetic saturation solubility is greater than thermodynamic equilibration solubility, this leads to an increased concentration gradient at membranes and thus subsequently to higher penetration or permeation through membranes [32, 33, 82].
4. High penetration through membranes is also favoured by high adhesion to biological membranes of nanocrystals [83-86].
5. According to several authors, transcellular uptake of nanocrystals through epithelial cells is another reason for the enhancement of bioavailability [11, 87-89]. Nevertheless, Gao et al. [90] concluded that results are conflicting and confusing and no further clarification could be highlighted.
6. Nanocrystals can be administered by intravenous injection (nanosuspensions) and are able to efficiently reach the target tissue or organ with 100% bioavailability [91].

As a consequence of their great potential, nanocrystals have been developed to deliver drugs under different administration routes. The following paragraphs present several examples of the most important administration routes for which a nanocrystal formulation has been developed with an improvement in drug bioavailability.

### *3.1. Oral drug delivery*

Oral delivery is the first choice in drug therapy, because of safety, patient compliance, ease of production, and scalability, though the principal limitations are related to drug bioavailability. Nanocrystals may improve bioavailability through an increase in solubility and particle dissolution, and through an increased gradient concentration at membranes and adhesion to the gastrointestinal

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wall. Classically, for Class II drugs, the rate-limiting step is the drug dissolution, and nanocrystals have been proposed to solve this limitation.

One of the first works highlighting this concept was carried out on danazol, a poorly soluble drug exhibiting poor bioavailability, that was formulated as three different formulations: an aqueous nanosuspension (169 nm), a danazol-hydroxypropyl-$\beta$-cyclodextrin complex, and an aqueous microsuspension (10 μm). The AUC after oral administration in beagle dogs revealed that the nanosuspension and the cyclodextrin complex had similar levels of bioavailability, while the bioavailability of the microsuspension was lower. The better performance of the aqueous nanosuspension compared to the aqueous microsuspension was explained by the fact that former overcame the limited dissolution rate normally observed with conventional suspensions. The authors thus proposed nanoparticles as the appropriate formulation for dissolution-rate limited absorption [27].

Following this pioneering study, many others confirmed the effectiveness of nanocrystals in improving drug bioavailability after oral administration. More recent examples are indicated in this section. In a lutein nanosuspension prepared by high pressure homogenization, nanocrystals exhibited a 26.3 fold increase in saturation solubility compared to that of coarse powder. The *in vitro* release of nanocrystals delivered in pellets and hard gelatin capsules for nutraceutical use was 3–4 times greater than that of coarse particles [6].

Rutin nanocrystals prepared by lyophilization and incorporated into tablets exhibited a higher particle dissolution rate than microcrystal loaded tablets and tablets already on the market [79]. The main factor in the increased dissolution was the particle size reduction, as shown by particle size measurements achieved by photon correlation spectroscopy (PCS) and laser difractometry (LD).

Four nanocrystals batches (size range 80–700 nm) of coenzyme $Q_{10}$ were prepared without any surfactant or polymer by the solvent/nonsolvent method. The dissolution rate of coenzyme $Q_{10}$ increased as particle size decreased, and the increased bioavailability of coenzyme $Q_{10}$ nanocrystals after oral administration was confirmed in beagle dogs where $AUC_{0-48}$ was 4.4-fold greater than that of coarse suspensions [35].

Nanocrystals of apigenin, a bioactive flavonoid poorly soluble in water, were prepared by the supercritical antisolvent process. Nanocrystals (400– 800 nm) exhibited a more rapid dissolution rate than coarse powder. After administration of a single oral dose in rats, nanocrystals showed a significantly decreased $t_{max}$, a 3.6-fold higher peak plasma concentration ($C_{max}$) and 3.4-fold higher area under the curve (AUC) than coarse particles [8].

Fenofibrate, a lipophilic drug used in hypercholesterolemia and hypertriglyceridemia, and which is practically insoluble in water, was prepared as a nanosuspension through processing in a probe sonicator and subsequent freeze drying to transform it into a dry powder. A decrease in particle size significantly increased its saturation solubility. Pharmacokinetic studies conducted in white rabbits confirmed a 4.73-fold increase in relative bioavailability compared to the pure drug form [9].

Nanocrystals of bexarotene, a potent anti-tumor drug of poor solubility and bioavailability, were obtained under a method combining precipitation and microfluidization. The decreased particle size thus achieved afforded a significant increase in dissolution rate, with improved *in vivo* results in rats. The higher AUC and lower $C_{max}$ indicated that oral bexarotene nanocrystals significantly increased the bioavailability of this important drug and decreased its side effects. Nanocrystals administered through intravenous injection showed higher bioavailability because of the absence of first pass effect and enterohepatic circulation [10].

Nimodipine nanocrystals of different sizes (159.0, 503.0 and 833.3 nm) were prepared by a combination of microprecipitation and high pressure homogenization. The *in vitro* and *in vivo* behaviour were compared to Nimotop®, a commercially available formulation of nimodipine. Even if Nimotop® exhibited a higher dissolution rate than the three different nanocrystal batches, the bioavailability measured by the plasma concentration–time curves determined in beagle dogs was significantly higher for optimized nanocrystals (159.0 and 833.3 nm), than Nimotop® [92].

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The *in vitro/in vivo* correlation for nimodipine nanocrystals and Nimotop® was explained by the fact that portions of nanocrystals underwent macropinocytosis and caveolin-mediated endocytosis by enterocytes as intact nanocrystal forms, then bypassed the liver first-pass metabolism [11].

Similarly, no *in vitro/in vivo* correlation was found in the case of itraconazole solid oral nanocrystals compared to the Spranox® formulation. Results showed rapid dissolution of nanocrystals, but this behaviour was not confirmed *in vivo* in a rat model [93]. The higher transit time of itraconazole from nanocrystals favours the rapid entrance into the small intestine where itraconazole is less stable, due to the pH at which the drug is less soluble. In Spranox® the dissolution occurs from the surface of the sugar beads, which presumably exhibit longer transit times in the stomach compared to nanocrystals. The highly concentrated solution in the stomach that is actually formed with the Spranox® formulation can stabilize the solution when it enters the small intestine. The stomach thus serves as a reservoir from which the highly concentrated solution can be delivered to the small intestine and be absorbed.

### *3.2. Intravenous drug delivery*

Due to their particle size, nanocrystals (nanosuspension) have the great advantage of being intravenously injectable, reaching 100% bioavailability. Nanocrystals in the range of 100-300 nm can be injected intravenously without any unwanted effect, such as obstruction of small capillaries. Consequently, nanoparticles circulate in the blood stream and dissolve according to their dissolution properties, and then are able to reach the target tissue.

One of the most powerful applications of intravenous injection of drug nanocrystal suspensions is the delivery of anticancer drugs [29, 30], because nanocrystal formulations permeate tumor tissues more effectively and are retained longer than other kinds of formulations [94, 95].

But, the formulation is also important because it affects the efficacy of the anticancer treatment. For example, Liu et al., [12] formulated paclitaxel nanocrystal in presence of D-R-tocopheryl polyethylene glycol 1000 succinate as a surfactant to stabilize the nanocrystals. Those nanocrystals exhibited a variety of benefits including high drug loading capacity, high stability, and sustained release. Most important, the surfactant was responsible for successfully reversing the multidrug resistance generally observed in presence of paclitaxel formulations, in both *in vitro* and *in vivo* experiments.

Another potent breast and lung anticancer drug, asulacrine, an inhibitor of topoisomerase II, was formulated as nanocrystals by high pressure homogenization and subsequent lyophilization. The pharmacokinetic studies after intravenous administration in mice showed good AUC in liver, lung and kidney, and thus it was possible to predict an accumulation of the drug in some body compartments [91].

In a study on paclitaxel nanocrystals, Gao et al. [14] found that most of the injected dose prepared without surfactant coating was taken up by the liver (40%), while a minimal amount was present in the blood circulation and quickly eliminated. Thus, they treated the nanocrystal surface with polyethylene glycol (PEG) based polymers and examined the impact of coating on biodistribution, pharmacokinetics, and retention of the drug in the tumor tissue. The coating significantly enhanced blood circulation of the drug and accumulation in tumor tissue. This approach on nanocrystals is in agreement with that generally used for other nanoparticles (liposomes and polymeric nanoparticles), where the PEG hydrophilic layer decreased the macrophage uptake, prolonging the circulation time and thus the probability that nanoparticles can reach the target tissue [31].

Shegocar and Singh [96] modified the surface of nanosuspension of nevirapine, an antiviral non nucleoside reverse transcriptase inhibitor, with serum albumin, polysaccharide and polyethylene glycol to enhance its targeting potential. The coated antiretroviral drug accumulated in various organs of rat differently than the plain drug solution when administered intravenously. Nanosuspension showed higher mean retention time (MRT) values in brain, liver and spleen than the plain drug.

An improvement in drug saturation solubility and dissolution rate of oridonin with the respect to commercial formulation was possible using a nanosuspension prepared by high-pressure

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homogenization in presence of Pluronic® F68, Brij® 78, PVP K25, sodium dodecyl sulphate, or lecithin as stabilizers [33]. The saturation solubility of nanocrystals was far higher than that of commercial oridonin and oridonin physical mixtures with stabilizers. This strongly impacted the drug dissolution rate.

Fluorescent molecules have been used with nanocrystals in hybrid formulations to be applied in both therapy and diagnosis. Interestingly, Zhao et al. [16] integrated a guest fluorescent substance into the crystal lattice of a poorly soluble anticancer drug, paclitaxel, thus producing a hybrid nanosystem for the concurrent aims of tumor targeting and imaging.

### *3.3. Pulmonary drug delivery*

The pulmonary administration of drugs has proven highly successful not only for treating lung pathologies, but also for systemic action, because of the very large surface area available for drug adsorption, as well as the fact that the first pass metabolism effect is avoided. Fast onset and rapid particle deposition are other advantages.

However, there are also drawbacks to this route, such as limited dissolution of poorly soluble drugs, rapid clearance due to ciliary movements, less residence time and thus absence of prolonged effect, and unwanted deposition of particles in pharynx and mouth [17]. While administration of nanocrystals through this route can favorably exploit the advantages of nanocrystals and those of the pulmonary administration route [97], it has been proposed that pulmonary delivery of nanosuspensions would be best, as it would overcome these drawbacks [17].

Several successful formulations have been studied.

Nanocrystals of budesonide, a poorly soluble corticosteroid anti-inflammatory drug, were prepared by wet ball milling, and subsequently loaded into hyaluronic acid microparticles by the spray drying process. This system allowed for sustained budesonide pharmacological effects, that could be explained by the mucoadhesion of the hyaluronic acid on the pulmonary mucosa, its gelation, and then release of budesonide nanocrystals on the mucosa. The presence of mucoahdesive polymer overcame the mucociliary clearance and, consequently, prolonged the retention of the active substance in the lungs [18].

Baicalein nanocrystals were prepared by a combination of anti-solvent crystallization and high pressure homogenization. Nanocrystals exhibited significantly enhanced dissolution, confirmed *in vivo*: pulmonary baicalein was rapidly and extensively absorbed through the pulmonary mucosa, showing pharmacokinetics parameters identical to those obtained after the intravenous injection of this drug [19].

Nanocrystalline formulations of itraconazole were prepared by wet milling and compared to amorphous itraconazole prepared by an ultra-rapid freezing process. The particle surface area was comparable and the pulmonary delivery of amorphous itraconazole resulted in significantly higher systemic bioavailability than for the nanocrystalline itraconazole composition, as a result of the higher supersaturation, which increased the permeation [20].

### *3.4. Ocular drug delivery*

Drug delivery to eye tissues is particularly problematic because of generally poor drug bioavailability, drug instability, short residence time, poor drug solubility, low amount of aqueous humour, and loss of drug with tears. Nanocrystals (nanosuspensions) can enhance ocular drug permeation, favour controlled release, and promote targeting [17, 98], also guaranteeing fewer or more attenuated side effects than traditional formulations [99].

An example is offered by the forumulation of nanosuspensions of three practically insoluble glucocorticoid drugs (hydrocortisone, prednisolone and dexamethasone). They showed an enhanced rate and extent of ophthalmic drug absorption, and increased intensity of action of the drug. An increase in bioavailability has the important advantage of reducing the risks of adverse side effects associated with large doses of these drugs, such as cataracts, glaucoma, and optic nerve injury [100].

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The bioavailability of dexamethasone acetate was improved by increasing the saturation solubility and the residence time in the eye of an ophthalmic cationic nanocrystal formulation. The saturation solubility increased due to the nano-size of the crystals, while the residence time improved due to increased mucoadhesion by the cationic charge [34].

Nanocrystal suspensions of brinzolamide, a poorly soluble drug, were prepared to reduce the intraocular pressure. At both tested pHs 7.4 and 4.5, 100% of the drug dissolved in 1 minute. The lowering of intraocular pressure was investigated *in vivo* in rats and proved to be particularly effective [21, 101].

### *3.5. Dermal drug delivery*

Since 2006, with the first use nanocrystals to increase bioavailability of functionals upon skin delivery [102], many studies have sought to exploit nanocrystal formulations of poorly soluble drugs to increase their bioavailability by skin delivery [23, 103].

There are several major advantages to the nanocrystal dermal administration route. The higher particle surface of nanocrystals can enable increases in particle spreading and adhesiveness. Also, such formulations offer increased dissolution rate and increased molecule flow due to improved saturation solubility [24].

Vidlarova et al. [25] demonstrated that nanosuspensions can penetrate through the skin and accumulate in the viable epidermis, and investigated the mechanism of penetration of curcumin nanocrystals through the skin. Nanocrystals, produced through bead milling followed by high pressure homogenization, were formulated as low viscous nanosuspension at decreasing concentrations (2.0, 0.2, 0.02, 0.002%) and compared to nanocrystals in viscous hydroxypropyl cellulose gel. The authors found that low viscosity favors skin penetration and observed no significant differences in penetration profiles for formulations with higher nanocrystal concentration, while significantly lower penetration was exhibited for the 0.002% concentration. They concluded that the concentration gradient cannot totally explain the driving force for the skin penetration, in contrast to the generally accepted understanding. Vidlarova and colleagues proposed other mechanisms to explain their findings. Perhaps the nanosuspensions have higher kinetic saturation solubility than the nanocyrstals in the gel form. Or it may be that the nanocrystal concentration is able to adequately cover the skin surface, or it may be due to the large crystal surface area in contact with the lipid film of the stratum corneum. Nanocrystal skin penetration is therefore still debated, particularly because authors frequently do not further investigate this aspect in their studies.

For example, the importance of saturation solubility was highlighted by Colombo et al. [24]. The saturation solubility and dissolution rate were determined for nanocrystals of dexamethasone, ibuprofen, and tacrolimus. Nanocrystals, prepared by bead milling (approximately 300 nm), exhibited far higher saturation solubility compared to non-milled particles (1.0 μm), and nanomilling increased drug dissolution rates particularly for ibuprofen. In this study, the authors evaluated the effect of nanosize on the saturation solubility, but did not correlate the saturation solubility with transdermal penetration, or evaluate the potential of absorption of nanocrystals through the skin.

In another study, nanocrystals of the flavonoid apigenin, produced by a combination of bead milling and subsequent high pressure homogenization were formulated into a hydrogel for topical application. The nanometric particle size positively affected the ability of particles to coat the skin. The antioxidant capacity of apigenin was demonstrated by evaluating its antiradical scavenging activity. The fast release profile from nanometric range particles was also proven, but the effect on drug absorption was only supposed from previous data. No evaluation of possible penetration of nanoparticles through the skin was provided in this study [76].

Interestingly, Zhai et al. [82] reported a mechanism for nanocrystal penetration through hair follicles. Nanocrystals in suspension demonstrated good saturation solubility and thus good concentration gradient, which allowed increased penetration and accumulation in hair follicles.

## 4. PHYSICOCHEMICAL STABILITY

While designing, developing, and optimizing a nanocrystal process at the industrial level, it is necessary to address several issues, particularly those related to instability problems.

Particle agglomeration and amorphization, which are major issues for drug stability, are discussed in the following section. Table 3 summarizes the issues related to the development and production of stable nanocrystals.

**T**able 3: Summarization of the main instabilities affecting nanocrystals (nanosuspensions).

| MAIN INSTABILITY | TECHNIQUES PROVOKING THE INSTABILITY | REFERENCES |
|---|---|---|
| PARTICLE AGGREGATION | Wet comminution<br>Lyophilization<br>Hight pressure homogenization<br>Bead milling<br>Cavi-precipitation<br>Dehydration of the surfactant | [6, 7] [11] [15] [19] [24, 25] [28] [62] [71, 72, 76] |
| AMORPHIZATION | Spray drying<br>Lyophilization<br>Dry milling<br>Cryo-milling<br>Wet milling<br>Anti-solvent | [7] [13] [18] [20] [49] [62] [77] [104-109] |
| CRYSTALLIZATION | High pressure homogenization<br>Nanospray drying underwent | [19] [62] |

### *4.1. Particle agglomeration and stabilization*

The excess in Gibbs's free energy typical of particles of nanodimensions explains their tendency to agglomerate and/or aggregate to a less energetic state, and agglomeration/aggregation is a way to increase particle size, reduce surface energy, and minimize total energy [47]. The interaction between particles was described by Derjaguin and Landau (1941) [110] and then by Verwey and Overbeek (1946) [111]: their theory explaining the stability of colloidal suspension was termed the DLVO theory, an acronym formed by the first letter from each scientist's last name.

It quantitatively describes the forces interacting between charged surfaces and a liquid medium, and it combines the effects of van der Waals attraction and electrostatic interactions (attraction or repulsion).

Stabilization can be achieved by steric or electrostatic interactions or a combination of both. To stabilize drug nanosuspension, researchers exploit amphiphilic excipients (frequently surfactants) with hydrophilic and hydrophobic domains that favor the interaction between particles and wetting liquid. The high surface energy produced during the preparation of nanocrystals or nanosuspension generally requests the use of stabilizers to prevent agglomeration [112, 113].

An exhaustive list of stabilizers that can be used in drug nanosuspensions/nanoparticles stabilization was provided by Van Eedernbrugh et al. (2008) [112].

Stabilizers thoroughly coat nanoparticles and prevent agglomeration providing ionic or steric barriers [17].

The formation of this energetic barrier can be correlated to the presence of attractive or repulsive forces due to the presence of a surrounding molecules (surfactants, polymers, etc.) or solvent [112].

Several examples about the tendency to particle agglomeration and the use of stabilizers to prevent In a study of polymeric stabilizers for drug nanocrystal dispersions, Choi et al evaluated drug particle size by laser light scattering analysis, and found that it decreased with time to a limited value (steady state) that depended on polymeric stabilizers. To understand this phenomenon better, they measured the contact angle between seven drugs (ibuprofen, naproxen, prednisolone acetate, nifedipine, hydrocortisone acetate, itraconazole, anthracene) and 2 polymers (hydroxypropyl

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cellulose HPC and polyvinyl pyrrolidone PVP), and found that the surface energy of drugs and polymers was an important factor in influencing the steady state. They theorized that a correlation could be established by the chemical interaction between the drug and the stabilizer, in particular, that the stabilizer could be absorbed on the drug nanocrystal surface, thus providing a steric stabilization effect [114].

In another study, indomethacin and itraconazole were wet milled in presence of four types of stabilizers (poloxamer 188, poloxamer 407, polysorbate 80, polyethylene glycol PEG). The mean particle size and the Polydispersity Index (PI) were determined by Photon Correlation Spectroscopy (PCS). They found that lower the PI value, the more monodisperse the particles were. The morphological evaluation of particles was determined by Transmission Electron Microscopy (TEM). These techniques were selected to reveal the particle size reduction during time and the agglomeration tendency of the particles. The amphiphilic block copolymers (poloxamer 188 and 407) appeared to stabilize the nanoparticles more efficiently than the low molecular weight surfactant (polysorbate 80). Particle size reduction and stability were affected negatively by high viscosity solutions, such as poloxamer 188 for itraconazole and PEG for indomethacin and itraconazole. The stabilizers did not affect the crystalline state of drugs, as proven by Differential Scanning Calorimetry (DSC) and X-Ray Powder Diffraction (XRPD) [115].

Another study to limit aggregation was conducted on rutin nanocrystals [7] prepared as dried powder by liophylization in presence of 0.2% W/W% of sodium dodecyl sulfate (SDS) as stabilizer. Particle sizes and physical stability were determined by PCS and Laser Diffractometry (LD) immediately after preparation and after re-dispersion. Light microscope magnification by 1000 detected the nanosupsensions effectively. The authors were able to confirm that lyophilized nanoparticles re-dispersed in water without the aggregation that otherwise would have impeded adequate particle dissolution.

Quercetine nanocrystals prepared by three methods, namely, high pressure homogenization, bead milling and cavi-precipitation, were characterized for their physicochemical properties, and compared for stability after storage in refrigerator (4±2°C), at room temperature (25±2°C), and in an oven (40±2°C) for 180 days. All the nanocrystals produced by the three methods were crystalline, as confirmed by X-ray diffraction study. Nanocrystals produced by cavi-precipitation showed lower stability than those produced by the other two methods. In particular, recrystallization and agglomeration were responsible for the increase in particle size due to agglomeration tendency, according to the Ostwald ripening phenomenon. The authors explained that this instability was due to competition between the solvent (ethanol) used for the cavi-precipitation and the surfactant (Tween 80), which should have prevented particle agglomeration. The partial dehydration of the surfactant due to the presence of ethanol provoked the particle agglomeration (as confirmed by PCS, LD, Scanning Electron Micoscopy, SEM and the measurement of zeta potential by Dynamic Light Scattering, DLS) [71].

Nanocrystals of apigenin, a low water soluble flavonoid, were produced by a combination of bead milling and subsequent high pressure homogenization [76]. An apigenin macrosuspension was prepared in presence of Plantacare 2000® (alkyl polyglucoside) under high shear mixing. Dispersion was treated under bead milling and then under high pressure homogenization. This combination process made it possible to reduce particle size to approximately 150 nm, with a low polydispersity index, as proven by PCS and LD. The X-ray powder diffractometry showed that apigenin did not undergo amorphization, and that the process avoided the particle agglomeration or any Ostwald ripening instability, guaranteeing high formulation physicochemical stability. The combination of low energy (bead milling) and high energy (high pressure homogenization) processes together with the use of a stabilizer could explain the high physicochemical stability of apigenin nanocrystals.

Hesperitin flavonoid nanosuspensions were prepared by high pressure homogenization. Poloxamer 188, Inutec ®SP1, Tween 80, or Plantacare 2000® were used as stabilizers to prevent particle agglomeration. The ability to reduce particle size, detected by PC, LD and DLS, was approximately the same in presence of all stabilizers (approximately 300 nm), and no statistically significant differences in polydispersity index could be highlighted. Slight aggregation was observed in presence

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of Tween 80 (final particles were of 350 mean particle size). Inutec and Plantacare proved to be the best stabilizers, able to prevent any change in particle size and zeta potential even after 2 years of stability study, while the worst cases were those obtained using Poloxamer and Tween 80 [116]. The explanation was found in differences in the viscosity of the system: high viscous systems prevent aggregation and stabilize nanosuspensions.

These same conclusions were reached in another study in which nanosuspensions of resveratrol were produced by high pressure homogenization in presence of the same stabilizers used in the previous study (Poloxamer 188, Inutec SP1, Tween 80, or Plantacare 2000). Also in this study, the best stabilization was achieved in presence of 1% of Plantacare or Inutec [117].

Nanocrystals of caffeine, a medium soluble drug, lead to pronounced crystal growth [26]. Nanocrystals were prepared by pearl milling in presence of stabilizers such as PVP 40, Carbopol® 981, or Tween® 80, and in presence of water or ethanol as suspender liquids. In the study, it was proven that crystal growth, revealed by PCS and light microscopy, may be affected by several factors, such as the suspender liquid, the surfactant, and the steric stabilizer. It is possible that the latter two are absorbed into the crystal surface to different extents, thus affecting crystal growth. In this study, Carbopol® 981 was revealed as the best stabilizer even in its unneutralized form. In this case, the polymer can protonate and charge the newly created crystal surfaces of caffeine, which actually possesses several protonable groups, such as carbonyl or imine groups. The consequence is stabilization via electrostatic repulsion.

### *4.2. Amorphization and crystallization*

One of the classic approaches for enhancing drug bioavailability is the conversion of the crystalline drug to its amorphous form, because amorphous drugs are markedly more soluble than their crystalline counterparts [48, 118].

The thermodynamic and kinetic properties of the amorphous state, such as excess of enthalpy, entropy, and free energy, explain the highest solubility and dissolution rate of amorphous forms with the respect to crystalline one [36].

In general, the processes used to amorphize solids are fast solidification, solidification from melt, drying procedures (such as freeze or spray drying), grinding and compression [104-107].

Specifically, the most widely applied and developed pharmaceutical technologies to promote drug amorphization are solid dispersions and nanocrystal technologies. They share the same objective and similar issues but apply different technologies [4, 119].

In the past, even though pharmaceutical industries recognized the advantage of amorphous formulations for increasing drug bioavailability, they did not focus on the development and marketing of amorphous formulations because of problems related to physicochemical stability and the modification of the drug bioavailability during the drug product shelf life. More recently, though, the emergence of innovative strategies to stabilize amorphous drugs has driven an increase in patented amorphous formulations and new FDA-approved amorphous drug products [4].

In line with this trend, there has been an increase in the number of studies seeking to produce amorphous nanocrystals.

In general, the application of high pressures during production of nanosuspensions promotes solid amorphization, while low energy processes favor the achievement of completely crystalline structures [27, 120, 121].

Nanocrystals of nicergoline of a mean particle diameter of nearly 700-800 nm were prepared by a nanospray drying method [54]. Spherical nanocrystals were proven to be amorphous by DSC and XRPD, and stability tests revealed good physical stability of the amorphous nanocrystals stored at 3 °C for at least one year, confirming the results of a previous study that demonstrated that amorphous nicergoline had high physicochemical stability [37]. The observation that nanocrystals have a more rapid dissolution rate than native and coarse particles was explained by the particle dimensions and amorphous physical state of the nanocrystals.

The same nanospray drying procedure was applied to indomethacin (IDM) [62], along with two other methods, wet milling followed by lyophilization, and cryo-milling. Under the three methods,

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it was possible to recover pure particles of mean particle diameter ranging between 500 and 800 nm. During these three treatments, IDM underwent physicochemical modifications. Particles obtained under nanospray drying underwent partial amorphization and consequently crystallization under the metastable polymorphic form α. Thus, all the batches produced through this method were a mixture of amorphous and polymorphic forms α (the metastable one) and γ (the native and stable polymorphic form). IDM treated by the other two methods exhibited a different tendency to amorphization, but at the end only the γ form was present. The fastest intrinsic dissolution rates were observed for the batch prepared under nanospray drying and the one prepared after cryo-milling for 40 minutes. These results were influenced by the crystalline form and by a decrease in particle size, which also influenced the particle dissolution behavior.

Cryo-milling was also applied to glibenclamide [77] and particles of nearly 500 nm in mean diameter were obtained. A significant decrease in crystallinity degree was revealed by DSC and XRPD. Both the decrease in particle size and crystallinity degree concurred to improve the particle dissolution rate.

Trasi and Byrn (2012) [49] studied the physicochemical behaviour under cryo-milling of six different compounds with different properties to obtain an amorphous solid. Ketoconazole, ursodiol, indomethacin, griseofulvin, carbamazepine, and piroxicam were investigated by DSC, XRPD and hot-stage microscopy, which indicated that all the drugs underwent progressive amorphization during cryo-milling.

Cryo-milling does not always cause amorphization or change in crystallinity degree of drugs. In the case of both phenytoin and ibuprofen, no changes in physical state were observed by DSC and XRPD [109]. However, an important tendency to particle agglomeration was highlighted by PCS.

On the other hand, Kayaert and Van den Mooter (2012) [122] proved that milling cannot be considered the main cause of amorphization for nanosuspensions, and that water present in the nanosuspensions can act as a plasticizer that triggers recrystallization. In their study, they selected cinnarizine and naproxen as model drugs to produce nanosuspensions by ball milling, stabilized with hydroxypropyl methyl cellulose (HPMC 2910). Cinnarizine was selected as weak hydrophobic base, and naproxen as weak acid. They explained that the cause of amorphization can be found in the interplay between drug and stabilizer after drying. If a drug is soluble in the stabilizer in the solid state, an amorphous solid dispersion is formed at the interface.

Dry milling and wet milling (in presence of water) can give different results concerning the drug amorphization tendency, as demonstrated by two separate studies. In the first, dry milling of indomethacin at both cryogenic and room temperature resulted in an amorphous form, whether a stabilizer was used or not [108, 123].

In the second, indomethacin was subjected to wet milling under high pressure homogenization in presence of PVP K25 and poloxamer 407 as suspension stabilizers. As proved by infrared spectroscopy (IR) and modulated differential scanning calorimetry (MTDSC), indomethacin was only partially amorphized and the amorphous form was only present on the surface and for an amount lower than 1%. The authors concluded that the amorphization was prevented in presence of water, which inhibits the drug amorphization during wet milling [74].

### *4.3. Particle surface modification*

This review has included several examples of the use of excipients as stabilizers, but it should be noted the addition of stabilizers on the particle surface not only acts on the physical particle stabilization, but may also provide additional properties to the nanocrystals, modifying their bioavailability and pharmacological activity (Table 4).

For example, the residence time of nanocrystals in the gastrointestinal tract can be increased by improving the adhesiveness of nanocrystals to lumen with the incorporation of mucoadhesive polymers [124].

A number of studies describe the preparation of mucoadhesive nanocrystal formulations. Among them, Jacobs et al. (2001)[125] reported the formulation of a mucoadhesive hydrogel of different Carbopols containing nanosuspension of bupravaquone, an antibiotic frequently used in

AIDS patients. The oral bioavailability of bupravaquone is limited, and thus the mucoadhesive hydrogel promotes a prolonged retention time of the drug nanosuspension in the infected gastrointestinal tract.

In another case, orally administered nanosuspensions were modified on the surface through the absorption of mucoadhesive polymers such as chitosan and carbopol, which can increase the adhesion to the gut wall [126].

A mucoadhesive nanosuspension of hydrocortisone acetate in presence of Poloxamer 188 and chitosan chloride was produced by high pressure homogenization as layering dispersion in a fluidized bed process, followed by the application of an enteric coating to achieve controlled drug release. Pellets containing drug nanocrystals exhibited accelerated dissolution velocity and increased drug release compared to a reference formulation of microparticles [28].

When nanoparticles are administered by injection, the immune system recognizes them as foreign particles, and they are immediately opsonized by proteins and enzymes circulating in the blood, to be taken up by phagocyte cells (32, 34) [13, 127].

Thus, nanoparticles are distributed to the tissues and body compartments (particularly the reticulum endothelial system) according to their size, zeta potential, and composition. This phenomenon is termed as passive targeting and is exploited to target drug to specific compartments [38, 128].

The PEGylation of nanocrystals can prolong their circulation time, limiting opsonization and improving the therapeutic effect of the loaded drug. PEGylated nanocrystals of paclitaxel (PTX) were prepared using antisolvent precipitation augmented by probe sonication. The PEG molecules covered the surface of nanocrystals with an 11.54 nm fixed aqueous layer thickness (FALT). PEGylated nanocrystals showed significant tumor inhibition in breast cancer xenografted mice [65].

Active targeting, in contrast, is achieved by coating the particles with specific molecules able to interact with receptors or in general biological substrates [22].

Atovaquone can be safely and effectively used against *T. gondii in vitro* to treat toxoplasmic encephalitis, but the oral micronized solution shows poor bioavailability [129]. *In vivo* studies confirmed the capacity of nanosuspensions coated with sodium dodecyl sulphate to cross the blood brain barrier and permit the treatment of toxoplasmic encephalitis and other cerebral diseases.

In another study, serum albumin surface modified nanosuspensions of nevirapine, a non nucleoside reverse transcriptase inhibitor, were prepared by high pressure homogenization technique and stabilized with a surfactant solution. The biodistribution studies showed a higher accumulation of the nanosuspension in various organs of rat compared to the plain drug solution administered intravenously. Macrophage uptake showed the ability of coated nanosuspension to target the intracellular compartments, while higher drug levels were identified in spleen, liver and thymus without added cytotoxicity. The surface modification of the nevirapine nanosuspensions with serum albumin favoured the blood brain barrier crossing and accumulation in brain for more than 24 h [15].

Nanosuspensions of amphotericin B were produced by high-pressure homogenization in presence of different surfactants such as Tween 80, Tween 20, sodium cholate, Pluronic F127, Pluronic F68 all approved for intravenous injection. Results indicated that nanosuspensions coated with polysorbate 80 and sodium cholate increased drug brain delivery and inhibited the parasite *in vitro*. The *in vivo* results were less satisfactory probably because of the severe and rapid pathological effects of the parasite once it reached the brain [75].

**T**able 4: Stabilization and functionalization of nanocrystals (nanosuspensions) by means of different stabilizers and surface agents .

| TYPE OF STABILIZERS | MECHANISM | DRUG – ACTIVE COMPOUND | APPLIED TECHNOLOGY | REFERENCES |
|---|---|---|---|---|
| Ionic surfactants-charged polymers: | Electrostatic repulsion | Albendazole | Nanoprecipitation<br>Sonication | [130] |

| | | | | |
|---|---|---|---|---|
| sodium cholate, sodium deoxycholate, sodium lauryl sulfate, sodium dodecyl sulfate, sodium poly(ethylene imine), chitosan | (Prevent aggregation) | | High pressure homogenization | |
| | | | High speed homogenization | |
| | | | Milling | |
| | | Spironolactone | Wet milling | [131] |
| | | | High pressure homogenization | [132] |
| | | Curcumin | High speed homogenization | [133] |
| | | | High pressure homogenization | [134] |
| | | | Nanoprecipitation method | [135] |
| | | Nitrendipine | Precipitation + High pressure homogenization | [136] |
| | | Rutin | High Pressure Homogenization | [79] |
| Non ionic surfactant-polymers: celluloses, polyvinyl alcohol, polyvinyl pyrrolidone, polysorbates, pluronic, poloxamers, triblock-copolymers of polyoxyethylene and polyoxypropylene, hydroxypropyl methylcellulose | Steric barrier against aggregation (Prevent aggregation) | Albendazole | Nanoprecipitation | [130] |
| | | | Sonication | |
| | | | High pressure homogenization | |
| | | | High speed homogenization | |
| | | | Milling | |
| | | | Antisolvent precipitation method | [137] |
| | | Nitrendipine | Precipitation + High pressure homogenization | [138, 139] |
| | | Ibuprofen | Wet comminution | [140] |
| | | Naproxen | | |
| | | Prednisolone Acetate | | |
| | | Hydrocortisone Acetate | | |
| | | Anthracene | | |
| | | Itraconazole | Wet comminution/ Wet milling | [115, 140] |
| | | Indomethacin | Wet milling | [115] |
| | | Quercetin | High pressure homogenization | [141] |
| | | | Bead milling | |
| | | | Cavi-precipitation | |
| | | Apigenin | Bead milling + High pressure homogenization | [76] |
| | | Hesperitin | High pressure homogenization | [72] |
| | | Resveratrol | High pressure homogenization | [73, 142] |

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| | | | | |
|---|---|---|---|---|
| | | | Precipiation | [143] |
| | | Caffeine | Pearl milling | [82] |
| | | Curcumin | High speed homogenization | [133] |
| | | | Wet milling | [144] |
| | | | High pressure homogenization | [134] |
| | | | Nanoprecipitation | [135] |
| | | Dexamethasone | Wet milling | [145] |
| | | Diclofenac | Wet milling | [146, 147] |
| | | Pyrimethamine | Nanoprecipitation + High pressure homogenization | [148] |
| | | Nifedipine | High pressure homogenization | [149, 150] |
| Alkyl polyglucoside (Plantacare 2000®), hydroxypropyl methyl cellulose (HPMC 2910), polyvinyl pyrrolidone and poloxamer | Formation of an amorphous solid dispersion at the interface (Prevent amorphization) | Apigenin | Bead milling + high pressure homogenization | [76] |
| | | Cinnarizine and Naproxen | Ball milling | [122] |
| | | Indomethacin | Wet milling | [49] |
| | | Fenofibrate | Milling | [151] |
| Arginine, amphiphilic amino acid copolymers (Albumin, Leucin), vitamine E, polyethylene glycol succinate (TPGS), lecithin, hydroxypropyl methyl cellulose, sodium cholic acid | Biological active providing additional functions to nanocrystals (Promote of a stable formulation) | Nifedipine | High pressure homogenization | [152] |
| | | Naproxen | Wet comminution | [153] [154] |

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| | | | | |
|---|---|---|---|---|
| | | Amoitone B (anticancer agent) | High pressure homogenization | [39] |
| | | Prednisolone, Carbamazepine, Itraconazole, Baicalin, Cyclosporine | High pressure homogenization | [39] [155] |
| | | Paclitaxel | Antisolvent precipitation + sonication | [156] |
| | | Curcumin | High pressure homogenization | [157] |
| Chitosan (ammino group), Carbopol | Mucoadhesion (Promote absorption) | Hydrocortisone acetate | High pressure homogenization | [28] |
| | | Caffeine | Pearl milling | [23, 26] |
| | | Buparvaquone | High pressure homogenisation | [125] |
| Hyaluronic acid | Mucoadhesion by gelation (Promore absorption) | Budesonide | Wet ball milling | [18] |
| | | Paclitaxel | High pressure homogenizer | [41] |

## 5. CONCLUSION

In recent decades, nanocrystal formulations have emerged as a very interesting approach to improve the bioavailability of poorly soluble drugs. Initially, this was the principal interest in nanocrystals, but more recently, their application has evolved towards other goals such as sustained release, modified drug delivery, and drug targeting.

Thus nanocrystals are now used to achieve pharmacological objectives similar to those for which polymeric nanoparticles are employed. Like polymeric nanoparticles, they also have negative tendencies to instability, seen in aggregation or a change in the solid state of the drug, which must be addressed wisely in preparing effective formulations.

The full comprehension of these phenomena and methods for stabilization are fundamental in the design and production of nanosystems.

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