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

Pharmaceutical nanocrystals: production by wet milling and applications

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Nanocrystals are regarded as an important nanoformulation approach exhibiting advantages of increased dissolution and saturation solubility with chemical stability and low toxicity. Nanocrystals are produced in the form of nanosuspensions using top-down (e.g., wet milling or 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 the features, preparation methods, and therapeutic applications of pharmaceutical nanocrystals, highlighting their universality as a formulation approach for poorly soluble drugs.

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 to several biopharmaceutical problems. For example, in the case of oral administration, NCEs that have limited solubility and low dissolution rates in digestive juices can display low bioavailability, high fed/fasted state variability, high interpatient variability, retarded

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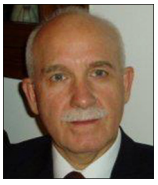

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onset of action, lack of dose proportionality, and local irritation [3]. Thus, it is evident that the limitation of poor solubility, which is one of the main reasons for the discontinuation of NCE development, makes their formulation challenging.

Previously, the pharmaceutical industry considered these compounds as highly risky development candidates. However, 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 to improve the solubility, dissolution rate, and subsequent bioavailability of drugs. These strategies include modifications of the drug properties at the molecular level (e.g., salt or prodrug formation, use of co-solvents, and complexation with cyclodextrins), the use of colloidal drug delivery systems (e.g., microemulsions and self-microemulsifying systems) or modifications of the drug properties at the particulate level (e.g., particle size reduction or amorphization) [5].

NPs 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 (NPs), there is no single definition of what a NP is. This might be because of the highly multidisciplinary nature of nanotechnology. The term 'nanotechnology' was first used by Norio Taniguchi in 1974, at the University of Tokyo, Japan, for any material in the nanometer size range [6].

According to the US 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, because many properties characteristic of the nanoscale (e.g., solubility, light scattering, and 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 [8]. Thus, the 100-nm limit is often considered constraining and, according to a more-inclusive definition, particles <1000 nm in each dimension (submicron particles) are designated as NPs [9]. The latter definition is applicable in the pharmaceutical field because particle size in the nanometer range can lead to increased dissolution rates because of 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 NPs, virosomes, nanocomplexes, and nanocrystals (Fig. 1 [11]).

Nanocrystals

Nanocrystals are nanosized drug particles. They 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}) <1 μm and a volume diameter 90% undersize (D_{90}) <2.5 μm to be classified as a nanosuspension (Fig. 2 [13–15]). Although 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, such as transmission electron microscopy [16,17]. The term 'nanocrystals', although implying that 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 because of changes from the crystalline to the amorphous form during processing [19,20]. In the strict sense, such an amorphous drug NP should not be called a nanocrystal. Recently, preparation of nanosized drug particles in the amorphous state has gained momentum because 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 NPs in the amorphous state (e.g., 'amorphous NPs' [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 and high permeability) and class IV (low solubility and 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 biorelevant 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 modifications might be preferable compared with nanocrystals for compounds belonging to DCS Class IIb and IV [25–27].

Yalkowsky and coworkers 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 the form of the 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, whereas 'brick dust' compounds display lower lipophilicity and higher melting points (m.p. >200 °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, whereas grease balls can be formulated as lipid-based formulations [30]. Thus, formulating drugs as nanocrystals should be mainly used as a solubility enhancement formulation approach to brick dust molecules rather than to grease balls.

From the different types of nanotherapeutics, we focus here on nanocrystals in the context of this review. Nanocrystals have a high drug loading (close to 100%) in contrast to matrix NPs comprising 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.

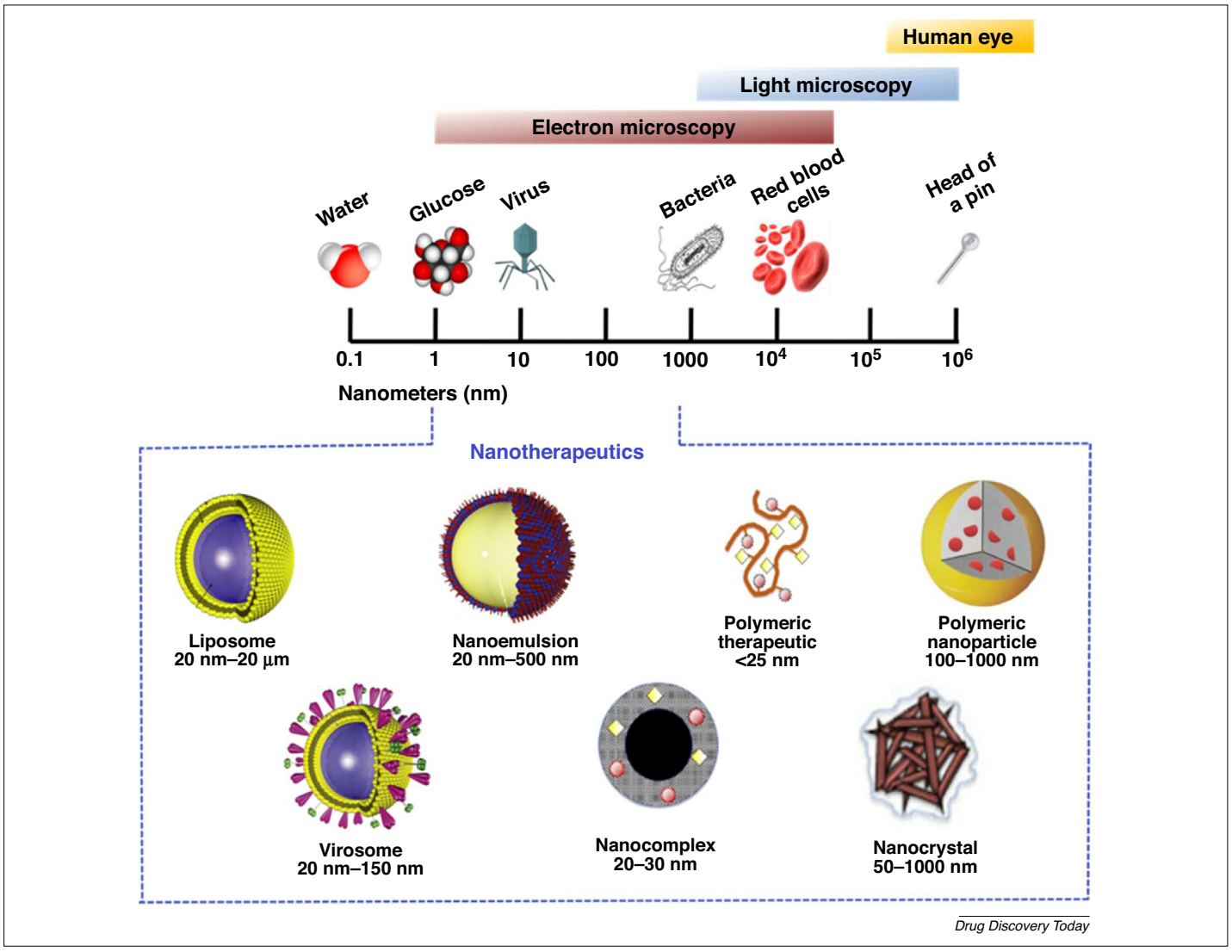

FIGURE 1

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

Advantages of nanocrystals in drug delivery

The increased saturation solubility and dissolution rate are the most important features of nanosuspensions. Saturation solubility for drug particles in the micrometer size range and above is a constant depending on temperature and dissolution medium; by contrast, 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 NPs resulting in increased dissolution pressure and, hence, drug solubility, as described by a modified Kelvin and Ostwald-Freundlich equation (Eq. (1))

$$ln\frac{S}{S_0} = \frac{2\gamma * V_m}{r * R * T} = \frac{2\gamma * M}{r * \rho * R * T}, \quad (1)$$

where S is the drug solubility at temperature T, S_0 is the solubility of infinitely 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, and 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 NPs lead to a more rapid dissolution, as described by the Nernst and Brunner equation (Eq. (2)):

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

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 ($C < C_s/10$).

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

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

where h_H is the hydrodynamic boundary layer thickness, k is a constant, V is the relative velocity of the flowing liquid against a

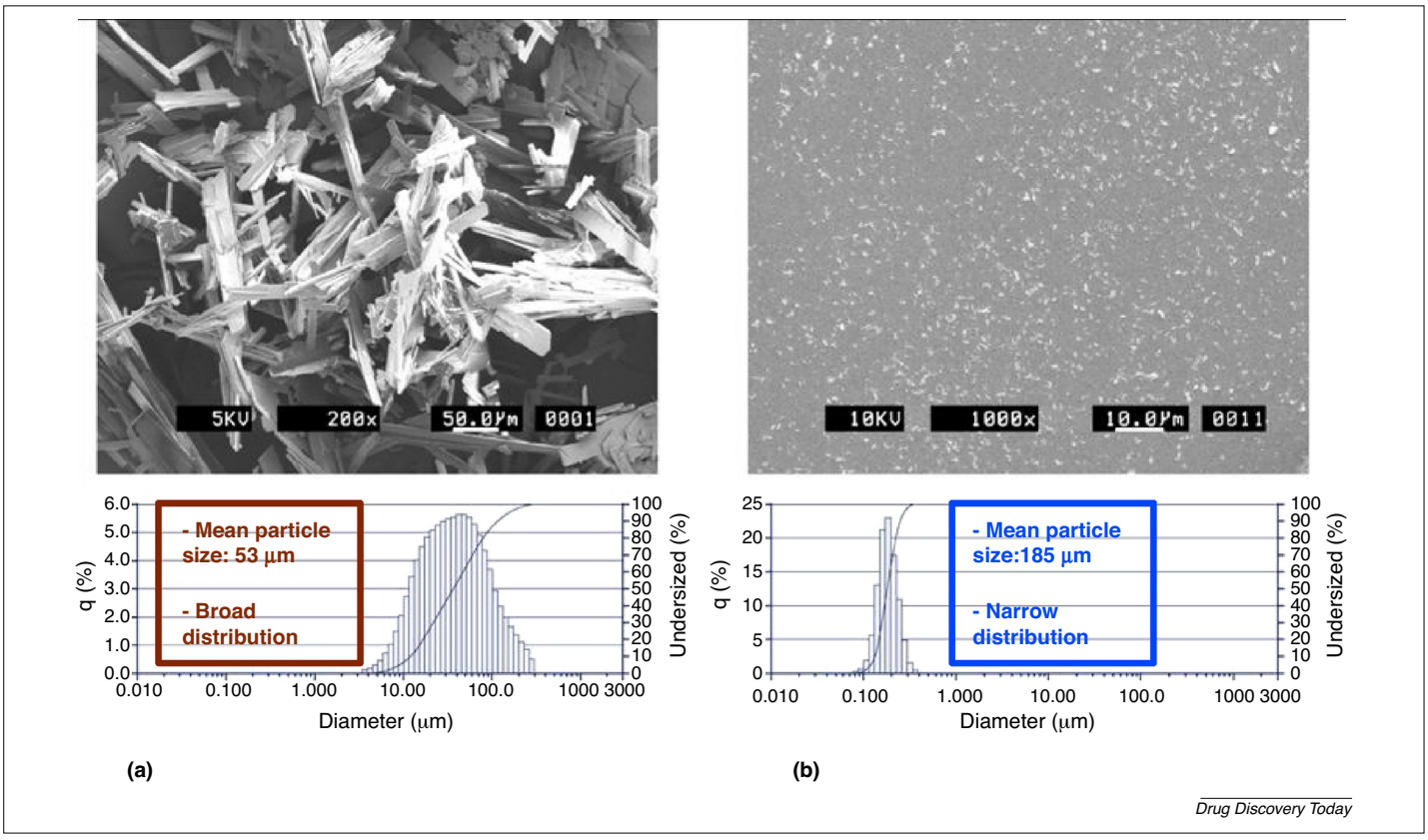

FIGURE 2

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 Ref. [15].

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, $(C_s–C)/h$, thus increasing the dissolution rate according to Eq. (2) [10].

Given 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 effects compared with micronized drugs [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, although it is not clear to what extent the saturation solubility can be increased solely as a 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, and turbidity). Based on their results, solubility increases of only 15% were measured, highlighting that solubility increases because of nanonization are relatively small. These measurements are in agreement with what would be predicted based on Eq. (1). Solid-state changes induced by particle breakage and increased surface wettability because of the presence of the stabilizer can also lead to enhancement of the 'apparent' saturation solubility and dissolution rate of nanosuspensions compared with micronized suspensions. Therefore, it is evident that the formulation and processing of drug nanocrystals are important for their *in-vitro* and *in-vivo* performances.

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 1 month. By 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 because they can extend the life cycle of an active pharmaceutical ingredient (API) after patent expiration [23]. Moreover, nanosuspensions can be used as formulations throughout the drug development process. Their quantitative and easy oral administration allows them to be used for preclinical animal studies [38], whereas, because of the scalability of their production (e.g., wet milling), formulation amounts ranging from a few milliliters up to a few liters can be generated. Small amounts are useful during preformulation stages, whereas larger quantities are required during toxicological and pharmacokinetic studies in animals and for clinical trials under good manufacturing practices (GMP).

All these characteristics have resulted in the rapid commercialization of nanosuspensions.

Stabilization of nanosuspensions

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

$$\Delta G = {}_{SL} * \Delta A - T * \Delta S. \quad (4)$$

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 to minimize the surface energy of the system.

For a nanosuspension to be stable, it must contain a third component, known as the 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 which was named after Boris Derjaguin, Len Landau, Evert Verwey and Theodoor Overbeek [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, whereas the well-solvated tail segment protrudes into the bulk medium. As two particles approach each other, the well-solvated segments of the stabilizer can interpenetrate. If the medium is a good solvent for the stabilizer molecules, the adsorbed segments on the particles cannot interpenetrate because the resultant desolvation is thermodynamically disfavored [41]. Compared with 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 nonaqueous 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 that 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 because of the electrostatic repulsion between particles and enhanced steric hindrance from the adsorbed polymers [43].

Various types of generally recognised as safe (GRAS) pharmaceutical excipient have been used as stabilizers of drug nanosuspensions. Detailed reviews and tables of 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 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 reported that there is a relationship between polymer affinity, solubilization capacity, and final particle size achieved. More specifically, they reported that there is an optimum stabilizer concentration for forming stable nanosuspensions with small particle sizes. When the stabilizer is present in the system in concentrations far above or below the optimum concentration, the nanosuspensions are prone to instability phenomena because of particle growth (Fig. 3). In the case of an insufficient amount of stabilizer, the surface of the nanocrystals is not completely covered by the stabilizer and, thus, particle growth occurs because of 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 because of 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 have attempted 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 that have a role in nanosuspension stability. They identified log *P*, melting point, and 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. However, other studies that investigated the stabilization of various drugs using different stabilizers at various concentrations reported no correlation between the physicochemical characteristics of a drug (e.g., molecular weight, melting point, log *P*, or solubility) and its ability to form a stable nanosuspension [49,50].

Formation of nanocrystals

Methods for the production of nanosuspensions can be categorized as top-down and bottom-up methods, depending on the starting material. In top-down methods, such as wet milling, high-pressure homogenization, and microfluidization, the starting material comprises larger solid particles than the resulting NPs 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 because the solvent in which the drug is dispersed, but not dissolved, is water for most poorly water-soluble drugs, making the top-down methods ecofriendly. This permits the formulation of many poorly soluble APIs, characterized as 'brick dust', suffering from poor

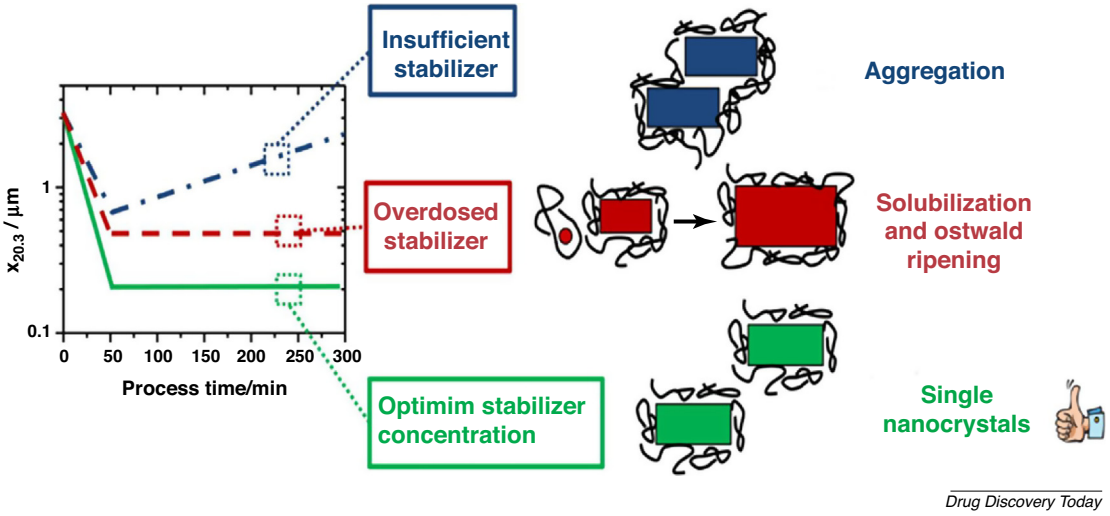

FIGURE 3

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 solubilization and increase of particle size due to Ostwald ripening. Adapted, with permission, from Ref. [46].

solubility in a 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 below, because it is the production method behind most of the marketed and developmental nanosuspension-based pharmaceutical formulations (Table 1).

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 occurs via 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 with 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 into stroke and collision. Wet milling is discussed and its application in drug nanonization considered in more detail below.

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 resulting 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, during 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 setup is important for determining the milling duration that should be selected to achieve particles of the desired fineness. Various mathematical modeling 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 modeling approaches extend from purely descriptive dynamic models to discrete element modeling, population balance models, and microhydrodynamic models. A detailed review of 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 mill used are rotor-stator and media mills.

Rotor-stator mixers/wet mills

Rotor-stator mixers comprise 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 because 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 that, apart from mixing, also lead to size reduction [55].

Wet media mills

The second type of mill 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.

TABLE 1

Nanocrystalline-based products approved by the FDA[a,b]

Trade name	Company	Active substance	Indication	Particle size reduction method	Route	Dosage form	Year
Avinza®	King Pharma	Morphine sulfate	Pain medication	WMM	Oral	Capsule	2002
Azopt®	Alcon	Brinzolamide	Ocular hypertension	WMM	Ocular	Suspension	1998
Cesamet®	Lilly	Nabilone	Antiemetic	Precipitation	Oral	Capsule	2005
Emend®	Merck	Aprepitant	Antiemetic	WMM	Oral	Capsule	2003
Focalin XR®	Novartis	Dexmethylphenidate HCl	ADHD	WMM	Oral	Capsule	2001
Gris-Peg®	Novartis	Griseofulvin	Antifungal	Precipitation	Oral	Tablet	1982
Herbesser®	Mitsubishi	Diltiazem	Hypertension	WMM	Oral	Tablet	2002
Invega Sustenna®	Johnson & Johnson	Paliperidone palmitate	Antipsychotic	WMM	Intramuscular	Suspension in prefilled syringe	2009
Megace ES®	Par Pharmaceutical	Megestrol acetate	Appetite stimulant	WMM	Oral	Suspension	2005
Naprelan®	Wyeth	Naproxen sodium	NSAID	WMM	Oral	Tablet	2006
Rapamune®	Wyeth	Sirolimus (rapamycin)	Immunosuppressant	WMM	Oral	Suspension, tablet	2000
Ritalin LA®	Novartis	Methylphenidate HCl	ADHD	WMM	Oral	Capsule	2002
Theodur®	Mitsubishi Tanabe Pharma	Theophylline	Asthma, COPD	WMM	Oral	Tablet, Capsule	2008
Tricor®	Abbott	Fenofibrate	Hypercholesterolemia	WMM	Oral	Tablet	2004
Triglide®	SkyePharma	Fenofibrate	Hypercholesterolemia	HPH	Oral	Tablet	2005
Verelan PM®	Schwarz Pharma	Verapamil HCl	Hypertension	WMM	Oral	Capsule	1998
Zanaflex®	Acorda	Tizanidine HCl	Muscle relaxant	WMM	Oral	Capsule	2002

[a] Based on Refs [16,23].

[b] Abbreviations: ADHD, attention deficit hyperactivity disorder; COPD, chronic obstructive pulmonary disease; HPH, high-pressure homogenization; NSAID, nonsteroidal anti-inflammatory drug; WMM, wet media milling.

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

Wet media milling equipment

Wet media milling equipment used for the production of nanosuspensions 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 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} and Retsch), most 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 in 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 suspension temperature on the resulting particle fineness.

Planetary ball mills are mainly used for the development of drug nanosuspensions on a laboratory scale because of their mechanical simplicity and versatility. Wet milling using planetary ball mills has been successfully used 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 mill equipped with up to 24 milling beakers of 0.05–1.0-ml individual milling chamber volumes. Scaling-up studies to a laboratory stirred media mill resulted in satisfactory comparability, indicating that a particle formulation optimized in a planetary ball mill can be transferred to other mill types used for the production of larger batch sizes. 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 because of a very high number of stress events per unit time and unit volume and

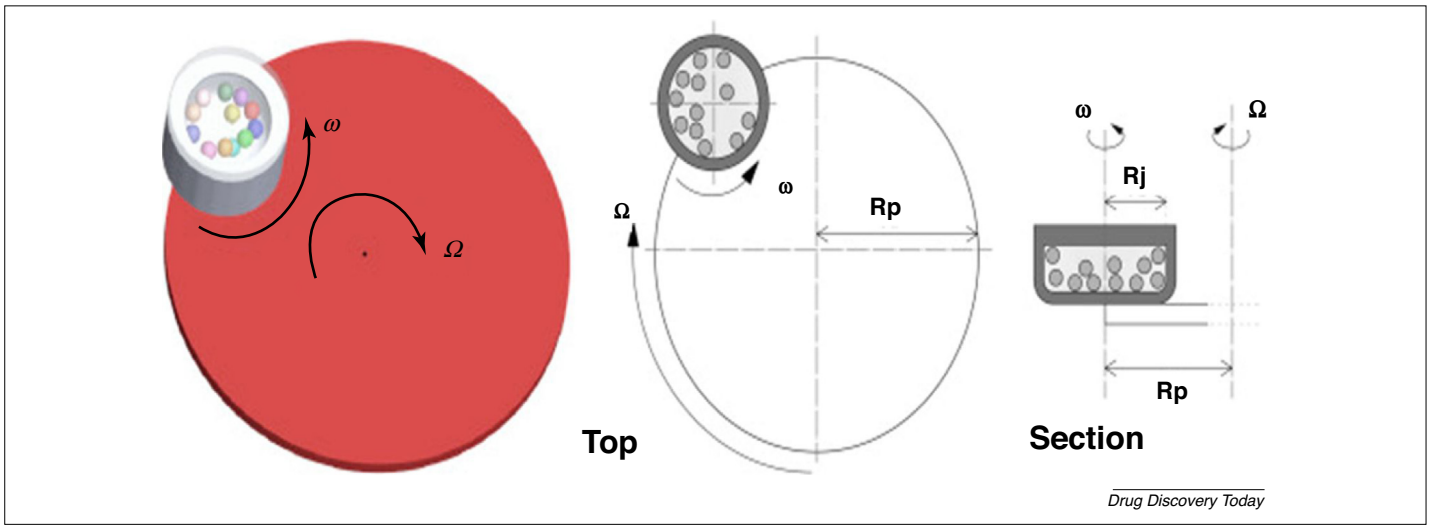

FIGURE 4

Schematic drawing of a planetary ball mill: (a) 3D 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 Ref. [56].

because of 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 8–20 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 <1 l to >1 m^3) and the stirrer geometry (e.g., disk or pin-counter-pin stirrer). Usually, this type of mill is equipped with a cooling system, allowing not only precise temperature control, but also processing of thermolabile compounds because 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. The batch mode is mainly restricted to the development of nanosuspensions at the laboratory scale. In the recirculation mode, a recirculation pump and a holding tank are added in the milling set-up (Fig. 5). The pump is used 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 operations, a receiving tank is also used, allowing the continuous withdrawal of product from the mill. There are two types of continuous mode: the multipass continuous and the cascade-continuous mode. In the multipass continuous mode, the suspension flows from the holding tank, through the mill, and into the receiving tank, whereas, 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 used in a continuous mode is a significant advantage of the process because the pharmaceutical manufacturing sector is moving towards the implementation of continuous processing strategies.

Applications of nanocrystals in drug delivery

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 used after the preparation of nanosuspensions. Spray and freeze drying (lyophilization) are the most commonly used techniques, whereas fluidized-bed coating, granulation, and pelletization 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 the pharmaceutical industry, have also been used [64]. It is important for the solid nanocrystalline-based formulations to retain their redispersibility (i.e., ability to reform NPs upon rehydration) because it is a prerequisite for their superior clinical performance. For this purpose, the addition of matrix formers (e.g., sugars) is a common strategy 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 to eliminate limitations related to the first commercially available formulation of sirolimus, which was 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]. By contrast, Rapamune® tablets exhibited a 27% increase in the bioavailability of the drug compared with the lipid-based solution and its ease of administration contributes to enhanced patient compliance [67].

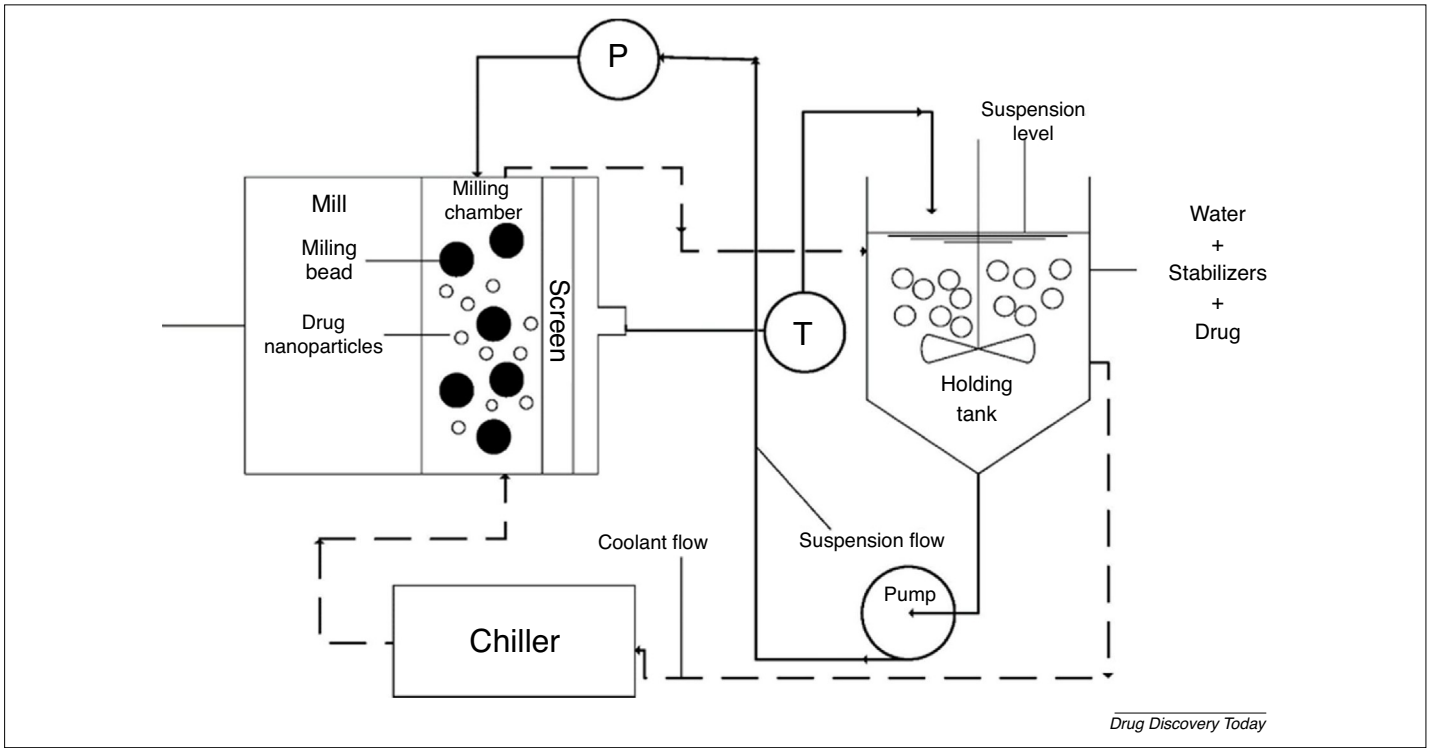

FIGURE 5

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

Emend® (Merck) is a nanocrystalline-based product of the antiemetic drug aprepitant, which was also developed using Elan's NanoCrystal® technology. It is formulated as capsules containing sugar beads coated with an aprepitant nanosuspension. Nanonization 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. Although the oral solution of megestrol acetate exhibited significant food effects, 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. Given that the patient population for this drug exhibits difficulties in consuming food, Megace ES® as a stable and non-viscous nanosuspension contributes to enhanced patient compliance [67].

Tricor® (Abbott) is a nanocrystalline-based formulation of fenofibrate for the treatment of hypercholesterolemia. The product is again based on Elan's NanoCrystal® technology and is available in the form of tablets. Launching Tricor® as a nanocrystalline formulation was part of the strategy of the company that involved the sequential launch of branded reformulations of fenofibrate to maintain a dominant market share years after generic competition was permitted [68].

Parenteral drug delivery

Via the parenteral route of administration (i.e., subcutaneous, intramuscular, intravenous (IV), intradermal, and intra-arterial injection), the drug can be administered directly into a blood vessel, organ, tissue, or lesion. Nanotherapeutics hold great potential for the 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 sterilization of these formulations using conventional methods, including gamma irradiation, filtration, and thermal sterilization [71]. Currently, several poorly water-soluble drugs have been formulated as nanocrystals for IV, intramuscular, and intraperitoneal administration [72]. For nanosuspensions intended to be administered IV, the particle size stability of nanocrystals upon storage is vital, and the content of particles >5 μm should be strictly controlled to avoid capillary blockade and embolism.

Regarding IV administration, a few studies have reported the development of nanocrystals as tumor-targeting drug delivery approaches. 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 tumor 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 mononuclear phagocytic system cells by *in-vitro* protein adsorption studies using 2D 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 with 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 nanonization of nevirapine significantly improved its *in-vivo* behavior 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 to be effective in controlling the acute symptoms of schizophrenia and delaying relapse of the disease. The formulation, as a nanosuspension, was also 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 that comprises an initial dosing and a maintenance dosing period. The concept behind the development of Invega Sustenna® is different compared with 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 its release [67]. That InvegaSustenna® is administered once-monthly is an advantage that gives increased product safety, tolerability, and, most importantly, improved patient compliance, compared with other antipsychotic drugs that require daily dosing.

Pulmonary drug delivery

Many of the advantages outlined above 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 that 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 occurs faster than drug absorption, as in the case of drugs with low dissolution rates, this can lead to a reduction in its bioavailability. Formulations comprising NPs 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, and 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 nebulized 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 nebulizers, solidification of nanosuspensions to respirable NP agglomerates (aerodynamic diameter between 1 and 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 NP agglomerates is 'an approach to harmonize 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 NP agglomerates. The NP agglomerates produced by this platform were found to exhibit enhanced aerosolization and dissolution performance while retaining 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]).

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 because of the very low ocular drug bioavailability (usually <5%). Research has focused on nanocarrier-based drug delivery systems (e.g., liposomes and polymeric micelles) because 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 has gained in popularity, attributed to the faster clinical development and commercialization of nanocrystals compared with 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 the 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. By contrast, the prolonged drug release 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 and/or surfactants that were screened as stabilizers during wet media milling, hydroxy-

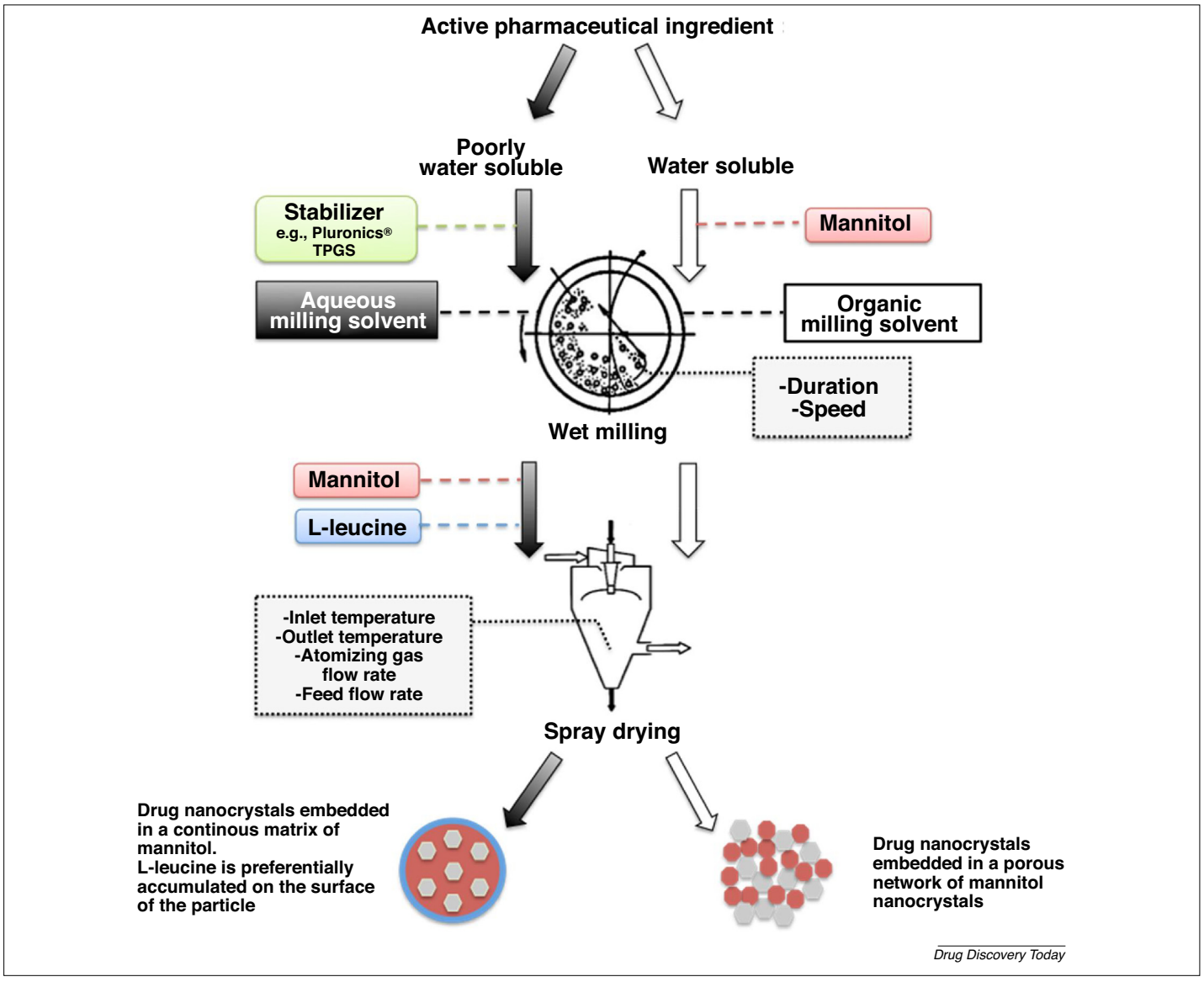

FIGURE 6

Preparation of respirable nanoparticle (NP) 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 NP agglomerates, by considering the physicochemical properties of the drug in question.

propyl methylcellulose was found to be the stabilizer of choice because 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).

Dermal drug delivery

Dermal delivery of nanocrystals is a route of administration that was not fully exploited until relatively recently, despite the advantages of nanocrystals, such as adhesion, fast dissolution, and increased penetration, that can be useful for dermal application. The development of nanocrystals for delivery to the skin was first 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 because the aqueous nanosuspension is mixed with the cosmetic product (e.g., cream or lotion).

Currently, apart from a 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 because of higher kinetic saturation solubility, and a low density of nanocrystals on the skin surface to cover the skin densely enough to a achieve a sufficiently large area of direct contact of the crystal surface with the lipid films of the stratum corneum.

Lai *et al.* [89] prepared nanosuspensions and nanoemulsions (oil-in-water) of tretinoin, an active compound 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 nanosuspension was found to favor drug accumulation into the skin (dermal delivery) and to minimize diffusion of the drug through the skin into the systemic circulation (transdermal delivery).

By contrast, a nanoemulsion was used to improve both dermal and transdermal delivery. Moreover, photodegradation studies, using ultraviolet (UV) irradiation of the formulations, revealed that the nanosuspension could improve the photostability of tretinoin compared with the nanoemulsion and the methanolic solution of the drug. Therefore, formulating tretinoin as a nanosuspension appears to be a useful formulation approach for improving both the dermal delivery and stability of the drug.

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 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. The chemical stability and low toxicity of nanocrystals resulting from their high drug loading are also beneficial aspects of this formulation approach.

Various methods have been investigated and patented for the preparation of nanosuspensions, which can be classified as top-down (e.g., wet milling and high pressure homogenization) 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 optimization 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, eye, and skin.

In conclusion, the number of nanocrystalline-based products already commercially available, together with the increasing number of scientific research papers 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 further in the near future.

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