Nanocrystals for Delivery of Therapeutic Agents

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Abstract

Clinical application of many emerging new chemical entities remains a herculean task due to poor aqueous solubility and bioavailability problems. Nanoscale orchestrations of solid state of such NCEs render faster dissolution rate, increased saturation solubility and enhanced bioavailability. Nanocrystals are crystalline particulate systems with dimensions less than 1000 nm. Unique surface properties, high loading capabilities, marked enhancements in bioavailability, lower fast/fed state variability, low incidence of side effects, delivery through various routes like enteral, parenteral, pulmonary, dermal etc., scope for active and passive targeting and wide range of technologies available for commercial applications offers potential platform for exploration of drug delivery using nanocrystals. It is predicted that nanocrystals would account for about 60% of all nanotechnology-based products with a market capture of 82 billion USD by 2021. Recent surge in marketed products and greater market capture amongst all nanoparticulate systems emphasizes the need for further development of nanocrystals. Exploring the potential of synchronized release with targeting could help in effective treatment of infectious diseases, pain-related disorders, and also aid in cancer chemotherapy. This chapter aims at providing a brief overview of formulation, preparation methodologies, stabilization techniques, characterization, evaluation, applications, biopharmaceutical aspects, safety and efficacy, and regulatory perspectives related to nanocrystals.

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DSC · Crystallinity · Polymer · Nanotoxicological classification · Crystallization · Precipitation

Abbreviations
AUC Area under the curve
PDI Polydispersity index

1 Introduction

High throughput screening has revolutionized drug discovery and development programmes, but has increased the risk of development of poorly soluble compounds, as high throughput screening hits are likely to have high molecular weight and LogP. Poorly soluble compounds lead to problems in in vitro and in vivo assays during preliminary screening and also pose a major financial risk in the drug development process (Di et al. 2012). Poor solubility of an estimated 75% drug development candidates is a major concern in drug discovery and development despite increasing costs of development (Di et al. 2009). Devising strategies to develop formulations for such BCS class II and IV (poorly water soluble) drugs has always been a major obstacle for formulation scientists (Gao et al. 2008). Poor solubility has been tailored using various approaches like crystal engineering (Blagden et al. 2007), amorphization (Van den Mooter 2012), micronization (Loh et al. 2015), prodrug synthesis (Stella and Nti-Addae 2007), cyclodextrin complexation (Jambhekar and Breen 2015), use of cosolvents, use of lipid vehicles and polymeric carriers (Mehnert and Mader 2001) etc. since long, with specific applications and occasionally with longstanding setbacks.

Various nanotechnology-based strategies like nanoemulsions, nanocrystals, polymeric micelles, lipid nanoparticles, dendrimers, and carbon nanotubes are being used to tackle poor solubility and bioavailability issues of BCS class II and IV drugs (Chen et al. 2011; Pathak and Raghuvanshi 2015). Nanocrystals constitute a unique group of all the nanotechnology-based products with majority of them designed for oral drug delivery. Nanocrystals are crystalline systems in the size range of 1–1000 nm with or without stabilizers. They act as a connecting link between crystalline form and amorphous form of a drug. Drug nanocrystals are comprised of 100% drug and do not contain any carrier/matrix materials like polymers or lipids. This differentiates nanocrystals from other nanoparticles. In the past few decades, extensive research is being carried out to develop new manufacturing technologies for nanocrystals, evaluate physicochemical properties of nanocrystals, understand and elucidate their stability and safety concerns. Benefits offered by nanocrystals in pharmaceutical field mainly include improved saturation solubility, enhanced dissolution velocity, improved bioavailability and the most important, patient compliance due to reduction in oral units of drug administered.

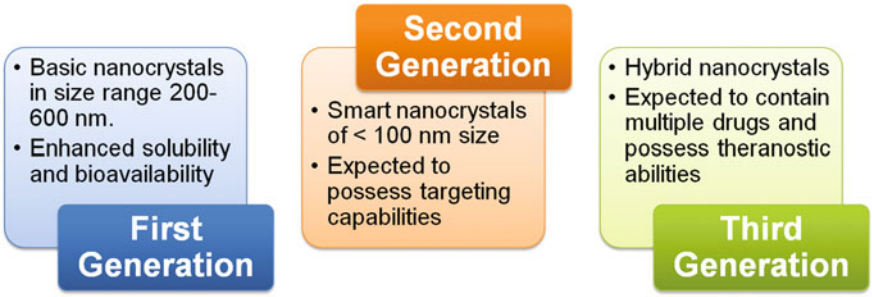

Fig. 1 Classification of nanocrystals

It is remarkable that these systems have entered pharmaceutical market in less than 10 years when compared to liposomes which took nearly 25 years to reach the market. Nanocrystals have demonstrated commercialization potential with a blockbuster product Tricor® whose annual sales are more than 1 billion $ in US with number of other products in pipeline that are about to enter markets in near future.

Tracking the progress of nanocrystals to date and anticipating future possibilities, the developmental journey of nanocrystals can be categorized into three generations as represented in Fig. 1. Literature available to date reports two generations of nanocrystals. First-generation nanocrystals are basic versions, mostly in the size range of 200–600 nm, intended for solving bioavailability and solubility issues of poorly soluble drugs (Patravale and Kulkarni 2004). Second-generation nanocrystals are smart crystals with a particle size less than 100 nm and possess targeting capabilities (Keck et al. 2008). Considering the remarkable progress achieved by nanocrystals during the past few decades, we forecast the development of a third generation nanocrystals representing hybrid systems containing multiple drugs and/possessing theranostic capabilities (Lu et al. 2015).

2 Advantages of Nanocrystals

Nanocrystal possesses some unique features like enhanced saturation solubility, improved dissolution velocity, enhanced bio-adhesiveness to cell membranes and cell surfaces which mainly helps in tackling many biopharmaceutical issues associated with poorly soluble drugs such as low bioavailability, large injection volumes, low dermal penetration and large propensity of side effects. Enhancement of saturation solubility by nanocrystal can be proven through Ostwald-Freundlich equation, which states that saturation solubility is inversely correlated with particle size, and found to be more pronounced as particle size is below 1 μm, as is the case with nanocrystal. However, enhancement of dissolution velocity can be explained from Noyes–Whitney equation. It can be easily confirmed that size reduction to nanometer scale leads to an increase in surface area and ultimately increase dissolution velocity as it is directly proportional to surface area. Enhanced bioadhesion

of nanocrystal can be explained because the particle size reduction to nano level helps in easy penetration into gastric mucosa. Various benefits offered by nanocrystals are depicted in Fig. 2.

Nanocrystallization as a solubilization strategy avoids use of solvents, surfactants, and oils. Of all the nanotechnology-based products, nanocrystals are reported to have highest drug loadings. Significant reduction in therapeutic doses is also observed due to enhanced bioavailability. Enhanced physical and chemical stability of drugs is seen when compared to amorphous forms and other nanotechnology-based products. Nanoscale crystallization helps in passive targeting through enhanced permeation and retention effect (EPR) and active targeting can also be achieved by conjugating with various peptides, antibodies, etc. Additionally nanocrystals are given "New Drug Product" status by USFDA and are very cost effective.

Fig. 2 Advantages of nanocrystals

3 Formulation

Formulation of nanocrystals involves a poorly soluble drug and a stabilizer. Optimal benefits of nanocrystallization are seen with drug molecules possessing high molecular weight (paclitaxel, sirolimus, etc.), high melting point (high crystal lattice energy like telmisartan, hydrochlorothiazide, etc.), and a solubility of less than 0.2 mg/mL (albendazole, celecoxib, itraconazole etc.), because the advantages gained due a smaller particle size are the highest with these types of compounds (Rabinow 2004). Brick dust drugs, which are very difficult to formulate can be easily formulated using advanced nanocrystal technologies (Chingunpituk 2007). BCS class II drugs with poor solubility and high permeability are ideal candidates for formulation of nanocrystals. Class IV drugs may not be ideal candidates for nanocrystallization, but recent reports reveal permeation enhancements using nanocrystals. Drugs with narrow absorption window would also be ideal for the development of nanocrystals as rapid dissolution of nanocrystals in the absorption window would enhance the bioavailability significantly.

Various methods have been explored for the producing drug nanocrystals. They are categorized as bottom-up, top-down, combinative, and miscellaneous approaches. Bottom-up approaches in which crystals are formed at molecular level as in precipitation, top-down approach where in larger micron sized are broken down to nanosized particles by milling or high pressure homogenization and combinative approaches employing both bottom-up and top-down techniques. In all the above processes, a larger surface area is formed increasing the total free energy of the system. Such systems are thermodynamically unstable and tend to agglomerate. This agglomeration tendency is opposed by the addition of stabilizers (Rabinow 2004). Various processes used for the preparation of nanocrystals are depicted in Fig. 3 (Van Eerdenbrugh et al. 2008b; Borchard 2015; Lu et al. 2015).

3.1 Bottom-Up Approaches

Bottom-up approaches include crystallization/precipitation methods. It involves addition of an anti-solvent to drug solution with or without stabilizer. Optimal control of process parameters to promote crystal nucleation and allow crystal growth in nanometer range is a pre-requisite for development of nanocrystals using this approach. This process is critical and can result in formation of polymorphs. The bottom-up approaches require the use of solvents that are usually difficult to remove completely. Presence of residual solvents is one of the major concerns with these processes as use of class 1 and 2 solvents may lead to harmful effects and organic residues present may lead to physical and chemical instability. In addition, needle shaped particles are usually produced in bottom-up approaches due to rapid growth in one direction. This tends to influence the physical stability of the

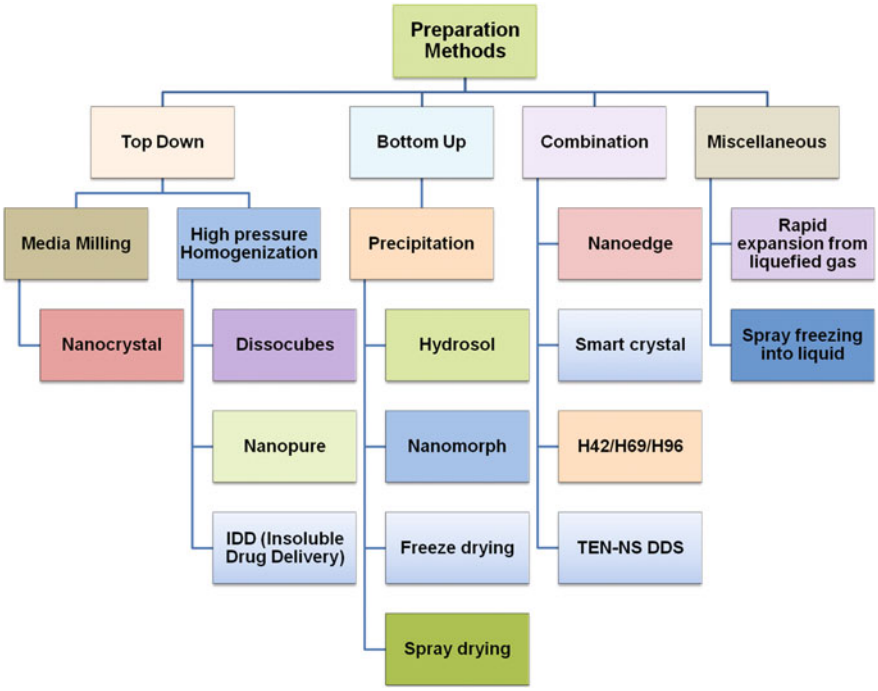

Fig. 3 Technologies used in the preparation of nanocrystals

nanosuspensions negatively (Verma et al. 2009). However, these methods are easier to process on large scale and are suitable for hydrophobic drugs. These methods involve crystallization, filtration and drying of nanocrystals, where input of mechanical energy is minimized compared to top-down methods. Besides conventional crystallization methods, latest technologies operating through high-gravity, supercritical fluids, ultrasonics, cryogenics and microemulsion templates are also utilized for crystallization of the drug nanocrystals. No method is universal, an appropriate choice of crystallization method is vital for the successful production of drug nanocrystals. Crystallization/precipitation process is mainly used. It is an instantaneous process with rapid nucleation kinetics. Mixing is crucial in such processes for determining supersaturation distribution which further determines the particle size distribution. Weakly acidic or basic hydrophobic drugs are ideal candidates for reactive crystallization. Addition of neutralizing solutions (strongly acidic or basic) decreases the solubility inducing crystallization. This method is relatively unexplored. Nanocrystals of few drugs, like crystals of itraconazole (Rabinow et al. 2007) and azithromycin, were obtained using this method, with an average size of 279.3 and 413 nm respectively.

3.2 Top-Down Approaches

Top-down approaches include media milling and homogenization which helps in production of nanocrystals using mechanical forces. These methods have been successful with few FDA approved commercial products on the market. These methods use high energy or pressure to achieve nanosized crystals. They are time consuming with intensive energy use and introduce impurities due to abrasion. Particle size control is inadequate and generates electrostatic effects (Van Eerdenbrugh et al. 2008).

3.2.1 Media Milling

Media milling using high-shear media or pearl mills is being used since long times for the production of nanocrystals. In media milling, the milling chamber is charged with the milling media (zirconium oxide, glass or highly cross linked polystyrene resin), formulation components and then operated at very high-shear rates. Nanosized crystals are produced by the shear forces produced due to impact of the milling media with the drug (Merisko-Liversidge and Liversidge 2011). Drugs with poor solubility in aqueous and organic media can be easily processed using media milling. Scale up is easy with little batch to batch variation and narrow particle size distribution. Contamination due to erosion of milling material is a major problem associated with this technology and this was significantly reduced by the introduction of polystyrene resin beads (Jia 2005). The Nano-crystals® technology developed by Elan Corporation was a core development in the commercialization of nanocrystal products. Nanomill® system was introduced by the same company for lab scale applications. Many products like Verelan PM®, Rapamune®, Focalin XR®, Avinza®, Ritalin LA®, Herbesser®, Zanaflex™, Emend®, Tricor®, Theralux®, Semapimod®, Theodur®, Naprelan® and Megace® ES were successfully commercialized using media milling process.

3.2.2 High Pressure Homogenisation

A high-pressure homogenizer is made up of a high-pressure plunger pump with a relief valve (homogenizing valve). The energy level required for the relief valve is provided by the plunger pump. The relief valve consists of a fixed valve seat and an adjustable valve. The gap conditions, the resistance and thus the homogenizing pressure vary as a function of the force acting on the valve. During the homogenization process, drug particles are fractured by cavitation, high-shear forces and the collision of the particles against each other. The drug suspension in the cylinder is passed through a very narrow homogenization gap. In the homogenization gap, the dynamic pressure of the fluid increases with a simultaneous decrease in the static pressure below the boiling point of water at room temperature. Hence, water starts boiling at room temperature, leading to the formation of gas bubbles, which implode when the suspension leaves the gap (called cavitation) and normal air pressure is reached again. The implosion forces are sufficiently high enough to break down the drug microparticles into nanoparticles (Krause and Muller 2001). Extensive use of energy, pre-micronization step before homogenization, high cost of instrument and

requirement of large number of homogenization cycles to achieve desired particle size are few disadvantages associated with this process. Micro-fluidizer technology (IDD-PTM technology), Dissocubes® technology (SkyePharma), or Nanopure® technology, (Abbott Laboratories) are various technologies developed using high pressure homogenization.

3.3 Combination Methods

Hybrid manufacturing methods were developed to reduce the time consumed for production of drug nanocrystals using regular methods. They are comparatively modern methods and couple crystallization process with high energy top-down techniques. Usually in combination methods, high energy via media milling, high pressure homogenization, ultrasonication, and high energy mixing is imparted post crystallization. Of all the methods, high pressure homogenization is the most popular method which is used in combination with other methods for production of most of the commercial products developed to date. Various drugs and nutraceuticals explored using combination methods are provided in Table 1.

3.3.1 Teniposide Nanosuspension Drug Delivery System (TEN-NSDDS)

TEN-NSDDS is the most recent combination process developed by He et al. In this approach, an anti-solvent sonication–precipitation method was used for the development of TEN nanosuspension. Initially, drug solution in acetone was added to anti-solvent under stirring at 1000 rpm for 10 min. The resulting precipitate was ultrasonicated using bursts for 3 s with a pause of 3 s for every two ultrasonic bursts, at a temperature of 4–8 °C. Residual acetone was removed under vacuum at 35 °C, for 12 h using rotary evaporation. Rod-like TEN nanocrystals with a size of 151 ± 11 nm and a narrow poly dispersion index of 0.138 was obtained. The obtained freeze dried TEN nanosuspensions were stable physically, for 3 months at 4 °C. When tested in rats with C6 tumors, the TEN concentrations in the tumor site was increased by 20-folds when compared to TEN solution at 2 h (He et al. 2015a).

3.3.2 ARTcrystal® Technology

Scholz et al. developed ARTcrystal® technology for producing flavonoid nanocrystals. It is a novel approach involving a rotor–stator pretreatment step with consequent high-pressure homogenization at low pressures for the production of drug nanocrystals. Various process parameters like size of starting material, flow rate, stirring speed, temperature, foaming effects, and valve position from 0° to 45° were studied in detail using an antioxidant rutin. One liter of nanosuspensions containing 5% rutin was produced in 5 min. Post optimization, a minimum pre-milling time of 5 min was recommended. Temperature was found to be a crucial variable affecting the yield and was suggested to be below 30 °C. A milling step with a rotor speed of 24,000 rpm and a flow rate (600 L/h, valve position of 45°) for 5 min at a temperature <30 °C could produce 1 L nanosuspension in 5 min in

Table 1 Drugs/Nutraceuticals explored using combination production technologies

Drug	Category	Technology	Process	Particle size (nm)	Advantages	Disadvantages	Reference
Teniposide	Anticancer	TEN-NSDDS	Precipitation + sonication	151	Small crystal sizes	Less stable product	He et al. (2015a)
Rutin, hesperidin and apigenin	Antioxidant	ARTcrystal®	Premilling + high pressure homogenization	411, 717 and 262 respectively	Faster and easily scalable	Larger particle sizes	Scholz et al. (2014), Scholz and Keck (2015)
Glibenclamide	Anti-diabetic	H96	Freeze-drying + high pressure homogenization	335	Finer particles and suitable for thermolabile products	Lengthy processing times	Salazar et al. (2012)
Apigenin	Antioxidant	CT	Pearl milling + high pressure homogenization	413	Finer particles	Possible contamination due to abrasion of beads	Al Shaal et al. (2010, 2011)
Ibuprofen	Anti-inflammatory	H69	Cavi-precipitation + high pressure homogenization	304	Improvement over Nanoedge®	Solvent residues	Muller and Moschwitzer (2006)
Glibenclamide	Anti-diabetic	H42	Spray drying + high pressure homogenization	236	Finer particles	Thermal degradation and amorphization tendencies	Moschwitzer and Muller (2006)
Itraconazole	Antifungal	Nanoedge®	Precipitation + high pressure homogenization	581	Smaller crystals compared to individual processes	Crystal growth during process and solvent residues	Kipp (2004), Kipp et al. (2003)

continuous circulation mode (Scholz et al. 2014). The proposed method is a fast and an economical process in which initial high-shear stress and subsequent cavitational forces (due to high pressure homogenization) are applied onto the crystals, thus achieving smaller crystal sizes in less amount of time when compared to traditional high pressure homogenization. Mean crystal sizes obtained using this process are in the range of 300–700 nm. Nanocrystals of various antioxidants like rutin (Scholz et al. 2014), hesperetin and apigenin (Scholz and Keck 2015) were successfully produced using this technology.

3.3.3 Combination Technology

Combination technology is a new development to classical bead milling, also known as smartCrystals technology. It consists of bead milling as a pretreatment with subsequent high-pressure homogenization. Shorter pretreatment times are needed in comparison to classical bead milling. Bead milling is carried out to achieve mean particle size of 0.6–1.5 μm followed by 1–3 cycles of high pressure homogenization at reduced pressures. Homogeneity of the intermediate blend obtained post pretreatment helps in reducing the cycle number and operating pressures. Pilot scale up at 3 kg level was successfully carried out achieving a mean particle size of 400 nm. Obtained formulations were stable up to 6 months at 4 °C, room temperature and 40 °C (Al Shaal et al. 2010). Apigenin nanocrystals for commercial applications were successfully developed using this technology. Nanocrystals with a mean size up to 396 nm and low PDI were developed using combination technology (Al Shaal et al. 2011).

3.3.4 H42/69/96 Technologies

These technologies were developed by Moschwitzer et al. exploring the potential of spray drying, freeze-drying and cavi-precipitation in combination with high-pressure homogenization for the production of nanocrystals (Moschwitzer and Muller 2006; Salazar et al. 2013). H42 technology was the initial development in this series combining spray drying with high-pressure homogenization. During the process, organic solution of the drug is added to aqueous solution with or without stabilizer followed by high-pressure homogenization (20 cycles at 1500 bar). Glibenclamide nanocrystals with a mean particle size of 236 nm and spherical morphology were successfully developed using this process. Organic residuals and scope for formation of amorphous phase are the major setbacks of this method (Salazar et al. 2013; Moschwitzer and Muller 2006). H69 technology combines microprecipitation and high-pressure homogenization. In this technology, organic solution of the drug is pumped into the homogenizer gap and anti-solvent is added in controlled manner, by controlled pumping, just before reaching the gap. Once the micro precipitation is initiated, the formed particles are passed through the homogenization gap that subsequently undergoes cavitation. During this process, annealing is applied by high-pressure homogenization to prevent further crystal growth to micrometer range and transform amorphous/semicrystalline form into a more stable crystalline state. This process is controlled by regulating the flow and ratios (Muller and Moschwitzer 2006). Ibuprofen nanocrystals with high degree of

crystallinity and a mean particle size of 304 nm were successfully produced using this technology (Sinha et al. 2013). Another development in this line of combination process is H96 process. In H96 process, drug suspensions are freeze dried, re-dispersed and immediately homogenized using high-pressure homogenization (Moschwitzer and Lemke 2006). This process is comparable to that of spray drying in H42 process, but by employing freeze-drying the process is made more suitable for thermolabile drugs (Teagarden and Baker 2002). Efficient utilization of H96 process was successfully demonstrated by Salazar et al. (2012) comparing it to high pressure homogenization. By freeze-drying, the degree of crystallinity can change tremendously, varying from 7 to 68% depending on the solvent ratio (dimethyl sulfoxide/tert-butanol). Pretreatment using freeze-drying allowed formation of smaller crystals of 335 nm at lower pressures compared to 691 nm using traditional high-pressure homogenization. More efficient results were obtained with pearl milling followed by freeze-drying pretreatment (160 nm compared to 191 nm) (Salazar et al. 2012). Marked reduction in size was attributed to the formation of a less crystalline, porous and brittle intermediate.

3.3.5 Nanoedge® Technology

It was the first combination process to be developed for nanocrystal production combining a microprecipitation and high-pressure homogenization (Kipp et al. 2003). Precipitation and high-pressure homogenization occurs separately in this process. Additional annealing step promotes size reduction of the crystals eliminating amorphous structures and enhancing physical stability (Kipp 2004). Major drawback of this technology is presence of solvent residues and a larger size distribution compared to other combination technologies.

4 Stabilization

Most common problem associated with nanonization is the instability of particles, which tend to aggregate. This results into instabilities like flocculation or sedimentation that are a major hurdle in development of pharmaceutical nanocrystals. Time required for aggregation may vary from seconds to hours or days. Flocculation is a process where destabilized particles conglomerate to form large aggregate. Attraction forces like chemical bonding or van der Waals forces is found to be responsible for aggregation. This physical instability is found to be responsible for loss of solubility and dissolution advantages offered by nanocrystals. Aggregation occurs via three different mechanisms, perikinetic aggregation, orthokinetic aggregation, or differential sedimentation. Perikinetic aggregation is mainly related to the rate of aggregation, which is governed by the frequency of collision of particles and the cohesive bond formation during the collision. Differential sedimentation arises due to different settling rate of the particles due to different sizes and density. Lastly, orthokinetic aggregation is mainly related to occurrence of aggregation due to extensive collision while particles are transported through

colloidal solution. Aggregation can be seen at various stages (production, storage and dissolution) during the developmental process leading to crystal growth and inconsistent dosing. Hence, there is a need to stabilize nanonized particles. Stabilization is predominately achieved by electrostatic repulsion and steric stabilization. Electrostatic stabilization is achieved by the formation of an electrical double layer around nanocrystals by adsorption of ionic charges resulting into generation of repulsive forces. Ionic strength of the medium has a significant influence on the repulsive forces. Due to its low cost and simplicity, this method of stabilization has been widely used but it is applicable to aqueous medium and not effective in solid form. Alternative technique available to electrostatic mechanism is steric stabilization in which non ionic amphipathic polymer is attached or adsorbed on the surface of nanocrystals. These polymers are mutually repulsive and hence prevent aggregation of particles. Advantages offered by steric stabilization mechanism over electrostatic, includes stabilized particles are re dispersible, influence of ionic strength of medium is ruled out and formulation with high concentration of nanocrystals can be obtained. Ionic-polymers which display unique properties of both polymers and surfactants impart electrostatic repulsion (surfactant property) and steric stabilization (polymeric property) (Shete et al. 2014). Various stabilizers used in the development of nanocrystals are enlisted in Table 2.

4.1 Selection Criteria for Stabilizers

Extensive literature is available regarding relationship between stabilization efficacy and properties of stabilizers. Various parameters related to drug, stabilizer and dispersion medium should be carefully assessed before choosing the stabilizer (Shete et al. 2014).

4.1.1 Drug-Related Parameters

Solubility of drug in stabilizer has significant impact on stabilizer selection. It is suggested that stabilizer in which drug has minimum solubility is mostly preferred as Ostwald ripening will occur at the expense of smaller particles which solubilize

Table 2 List of various stabilizers used in nanocrystal development

Type	Examples
Polymers	Povidone, polyvinyl alcohol, polyethylene glycol, carboxymethylcellulose sodium, hydroxypropyl cellulose, hydroxyethyl cellulose, hypromellose, decyl glucoside, etc.
Surfactants	Sodium lauryl sulfate, docusate sodium, tween 80, poloxamers (188, 338, 407), D-α-tocopheryl polyethylene glycol succinate, etc.
Food proteins and biopolymers	Zein, polylactic acid, whey protein isolate, soybean protein isolate and β-lactoglobulin
Amino acids	Phenylalanine and leucine

rapidly and crystallize around large particles. Another important drug-related parameter is zeta potential. It is the electrokinetic potential of colloidal system. It measures the interaction between colloidal particles. Zeta potential is an indicator of stability of colloidal system, and as it increases electrostatic repulsion increases. For a colloidal system to remain stable, zeta potential should be ±30 mV. George et al. reported that drug and stabilizer with nearly similar log P will form a stable nanocrystal suspension (George and Ghosh 2013).

4.1.2 Stabilizer-Related Parameters

High molecular weight stabilizers are preferred because long chain length would help in overcoming the van der Waals forces of attraction. Enough steric repulsion is not offered by short chain lengths and stabilizers with short chain lengths tend to promote aggregation. Polymers stabilizers with molecular weight ranging from 5000 to 25000 g/mol are generally used in the preparation of nanocrystals. Studies reported the influence of hydrophobicity of stabilizers on stability, which concluded that hydrophobic stabilizers are suitable candidates for stabilization of nanocrystal of hydrophobic drug as they are easily adsorbed on drug's surface. Concentration of stabilizers in media have significant impact on stability of nanocrystal medium as an optimum concentration of stabilizer is required to completely coat/cover the drug surface for efficient steric repulsion and formation of a stable system. However, some literature pointed out that efficiency of stabilizer is lost when its concentration exceeds critical micellar concentration. Another important stabilizer related parameter that has significant influence on stability of nanocrystal is viscosity. Positive correlation between viscosity and stability has been found as per Strokes–Einstein equation. This equation postulates that high viscosity ensures colloidal stability by reducing diffusion velocity of drug molecules. Other stabilizer related parameters such as surface energy and particle-stabilizer affinity have also proved their importance toward stability of colloidal system of nanocrystal.

4.1.3 Dispersion Medium-Related Parameters

pH and temperature play a significant role in electrostatic and steric stabilization. pH of aqueous medium affects stability of stabilizer performance mainly for ionizable polymers. Temperature affects the affinity between nanocrystal and stabilizer and hence leads to destabilization of the system. Cooling or heating of colloidal system of nanocrystal may lead to flocculation. Furthermore increase in temperature may lead to alteration of dynamic viscosity and diffusion coefficient.

5 Characterization and Evaluation

Different parameters affecting the quality of nanocrystal products are classified based on the colloidal nature of nanocrystals, bulk colloidal drug suspensions, stabilizer and dispersion media interactions, particle-stabilizer and dispersion media interactions and presence of contaminants. Various properties like content, presence

of impurities, size range, morphology, solid state properties, and thermal behavior should be carefully considered and evaluated to develop a stable nanocrystal formulation. Stabilizer adsorption, dissolution, conformation and dynamics of interaction should be addressed carefully. While dealing with bulk suspensions, electrokinetics, rheological parameters, sedimentation and agglomeration tendencies should be appropriately evaluated (Borchard 2015; Juhnke and John 2014).

Particle size distribution and zeta potential These parameters can be obtained using photon correlation spectroscopy (PCS) (Gulari et al. 1979), laser diffractometry (Baudet et al. 1993) and coulter counter analysis (Hurley 1970). A polydispersity index (PDI) value of 0.1–0.2 signifies a narrow size distribution, whereas a PDI value greater than 0.5 indicates a very broad distribution. A minimum zeta potential of ±30 mv is recommended for electrostatically stabilized nanosuspension, while a zeta potential of ±20 mv is required for a combined electrostatic and steric stabilization.

Crystallinity and morphology The changes in the physical state and the extent of the amorphous content can be determined by Terahertz spectroscopy, X-ray diffractometry (XRD), differential scanning calorimetry (DSC), modulated-DSC and scanning electron microscopy (SEM).

Dissolution Various factors to be considered to understand dissolution outcomes are composition of formulation, shape of crystals, surface area, size distribution, exposed planes, surface chemistry, crystallinity, media exposure, storage conditions, etc. Dissolution can be carried out as per compendia requirements. Apart from the USP Apparatus II paddle method, various other methods like supernatant-assay or dialysis, in situ monitoring of drug particle size reduction by turbidity measurement, pressure separation by liquid chromatography or field-flow fractionation followed by HPLC or UV spectroscopy, monitoring particle dissolution by Dynamic light scattering or UV fiber optic spectroscopy, etc., are being used to understand dissolution of drug nanocrystals (Borchard 2015).

Toxicology studies Hydrophobic interaction chromatography (HIC) can be employed to determine surface hydrophobicity, whereas 2-D PAGE can be used for quantitative and qualitative measurement of protein adsorption post IV injection (Gao et al. 2008). Haemolytic tests play a vital role when considering nanocrystal formulations for IV application (Liu et al. 2010). Various animal models can be employed to study organ distribution and toxicity.

6 Biopharmaceutical Aspects

Nanonization as a formulation strategy would help in bioavailability enhancement of poorly soluble actives as a function of particle size. Nanocrystals can achieve faster T_{max} and higher C_{max} proportionally increasing AUC. Minimal fed/fast state variability is observed with nanocrystals. Recent literature reporting bioavailability enhancements by nanocrystallization are reported in Table 3.

Table 3 List of recently published nanocrystals along with their biopharmaceutical outcomes

Drug	Therapeutic application	Method	Stabilizer	Particle size (nm)	Biopharmaceutical outcome	Reference
Satranidazole	Anti-protozoal	High-pressure homogenization	Span 20, HPMC E-5	208.8	Nanocrystals exhibited twofolds enhancement in bioavailability	Dhat et al. (2016)
Protopanaxadiol	Anticancer	Anti-solvent precipitation	TPGS	90.44 ± 1.45	Decreased T_{max} and increased C_{max} and AUC of nanocrystals when compared to physical mixture	Chen et al. (2016)
Lovastatin	Anti-hypertensive	Sonoprecipitation and bead milling	Mannitol and glucose	503.2 ± 20.4	Sevenfold and fourfold increase in C_{max} of rod-shaped nanocrystals and spherical nanocrystals respectively	Guo et al. (2015)
Nelfinavir mesylate	Anti-retroviral	Ultra sonication	PVA and poloxamer 407	236 ± 19.23	PVA formulation had decreased T_{max} and increased C_{max} and $AUC_{0\text{-}24}$ compared to pure drug	Naga Naresh et al. (2015)
Paclitaxel	Anticancer	High pressure homogenization	Pluronic- chitosan (Pl-g-CH) copolymer	192.7 ± 9.2	12.6-fold enhancement in relative bioavailability was observed with nanocrystals compared to that of Taxol™	Sharma et al. (2015)
Hesperetin	Antioxidant	Spray drying	Mannitol	137.3 ± 90.0	Nanocrystals have shown 1.79 and 2.25-fold increase in C_{max} and oral bioavailability respectively	Shete et al. (2015)
Puerarin	Vasodilator	High pressure homogenization	Sodium dodecyl sulfate	229	4.47-fold enhancement in bioavailability was observed when compared to pure drug	Yi et al. (2015)
Lacidipine	Anti-hypertensive	Bead milling	SDS and HPMC E5	623	2.05-fold increase in $AUC_{0\text{–}24}$ h compared to marketed product	Fu et al. (2015)
Saquinavir	Antiviral	High pressure homogenization	Poly(sodium 4-styrenesulfonate)	205.9 ± 3.74	2.16 and 1.95-fold increase in C_{max} and AUC respectively compared to that of coarse crystalline SQV suspension	He et al. (2015b)
Mebendazole	Anti-helminthic	Wet milling	PVA	210	C_{max} and $AUC_{0\text{-}8}$ were increased by four and threefold respectively	Hashimoto et al. (2015)
Cefdinir	Antibacterial	Wet milling	Poloxamer 407	224.2 ± 2.7	Threefold increase in oral bioavailability was demonstrated	Sawant et al. (2015)

7 Applications

7.1 Cancer Chemotherapy

To date, cancer remains as one of the most life-threatening disease resulting in 8.2 million deaths. A 45% raise in cancer related deaths is projected by 2030 as per WHO reports. IV administration is still preferred route for cancer chemotherapy due to poor solubility and limited oral absorption of most anticancer therapeutics. No significant improvements in this situation are expected as > 40% of cancer therapeutics in development display poor aqueous solubility. In the said scenario, nanocrystals with their unique features, as discussed earlier, would offer a potential platform for the development of safer and effective formulations for cancer chemotherapy. Improved pharmacokinetics and biodistribution can be expected due to uniform and stable physical nature of nanocrystals (Lu et al. 2015). Passive targeting can be expected through EPR effect and active targeting can be achieved by ligand conjugated nanocrystals (Wang et al. 2016; Pawar et al. 2014). Ye et al. recently developed injectable nanocrystals of brick dust drug niclosamide using wet media milling. Tween 80 was used as stabilizer achieving an average particle size distribution of 235 nm. Pharmacokinetics of nanocrystal formulations at a dose of 2 mg/kg were comparable to that of drug solution for anticancer effects in EC9076 cell line (Ye et al. 2015). Ntoutoume et al. developed cyclodextrin-cellulose nanocrystal complexes of curcumin and have shown enhanced cytotoxicity against PC-3, DU145, and HT-29 cell lines (Ntoutoume et al. 2015). Dong et al. developed injectable nanocrystals of anticancer agent SNX-2112 using wet media milling technique. Poloxamer 188 was used as a stabilizer and the particle size was 203 nm. Drug nanocrystals were rapidly absorbed showing comparable pharmacokinetics to drug-cosolvent system. Plasma concentrations, systemic clearance, distribution in heart, lung, kidney and intestine were comparable to that of cosolvent formulation. Accumulation of drug in liver and spleen was observed during initial 1 h due to particulate uptake (Dong et al. 2015). Pawar et al. prepared docetaxel nanocrystals using high-pressure homogenization employing pluronic F-127 as stabilizer. Nanocrystals have shown enhanced G2-M arrest when compared to the free drug and Taxotere® formulation. Enhanced safety of drug nanocrystals compared to the marketed formulation was successfully demonstrated by acute toxicity studies and hemolytic tests (Pawar et al. 2015). Growing literature suggests safety and efficacy of nanocrystals especially in cancer chemotherapy when compared to existing products. This opens potential avenues for the development of nanocrystal based delivery systems for cancer chemotherapy.

7.2 Targeted Drug Delivery

Nanocrystals offer potential platform for targeted drug delivery as their surface properties and invivo behavior can be easily tailored. Fuhrmann et al. have

reviewed targeting possibilities and limitations of injectable nanocrystals. Numerous possibilities for surface orchestration of nanocrystals provide enough scope for enhancing cellular uptake and tumor accumulation. Sub 100 nm size particles are known to penetrate tumors, which can be achieved by nanocrystals. Smart nanocrystals and hybrid nanocrystals which are in sub 100 nm range could thus find potential applications in targeted drug delivery. Modulation of drug release and identifying stimuli responsive stabilizer coatings can help in development of hybrid nanocrystals which can accumulate in disease sites. In addition, conjugation strategies would offer active targeting as seen with other nano carriers (Fuhrmann et al. 2014). Composite nanocrystals of gemcitabine and magnetite resulted in enhanced tumor accumulation providing stimuli responsive delivery through magnetic activation (Arias et al. 2008). Co-administration of tumor-penetrating peptides along with anticancer drugs may help in increasing vascular and tissue permeability leading to increased accumulation of drug at tumor site (Sugahara et al. 2010). Dong et al. synthesized folic acid conjugated cellulose nanocrystals for targeting folate receptor positive cells which are over expressed in breast, colon and ovarian cancer etc. Uptake of the nanocrystals was dependant on the type of cells. In DBTRG-05MG and C6 cells, nanocrystals were internalized via caveolaemediated endocytosis whereas in H4 cells, they were internalized via clathrin-mediated endocytosis (Dong et al. 2014). Wu et al. synthesized magnetic bioceramic hydroxyapatite (mHAP) nanocrystals by wet chemical precipitation process. mHAP nanocrystals were conjugated to hyaluronic acid to achieve targeting using PEG spacer arm. Hyaluronic conjugation helped in targeting MDA-MB-231 cell whereas superparamagnetic properties of nanocrystal composites helped in achieving intracellular hyperthermia for effective tumor eradication (Wu et al. 2016). Li et al. developed folate-chitosan conjugated nanocrystals on bexarotene using precipitation-high pressure homogenization method with a mean particle size of 631.3 ± 2.7 nm. Nanocrystals have shown threefold increase in AUC and 1.5-fold increase in Cmax when compared to drug suspension (Li et al. 2016). Nanocrystals were also reported to enhance drug delivery to brain. Chen et al. reported that surface modification of nanocrystals with efflux inhibitors and functional stabilizers helped in enhancing drug accumulation in brain (Chen et al. 2016). Combination of nanocrystals with various other ligands and functional materials can thus create new platforms for targeted drug delivery (Boles et al. 2016).

7.3 Theranostic Applications

A theranostic platform involves combination of diagnosis and subsequent therapy. From a material perspective, nanocrystals offer a potential for theranostic applications as multiple functionalities can be combined in one nanocrystal. Combining imaging agents with the host nanocrystals of anticancer agents will help in simultaneous tumor therapy and bio-imaging. Evolving generation of nanocrystals

called hybrid nanocrystals possess theranostic capabilities. Inorganic nanocomposites based systems provide a good platform for theranostic applications. Preparation methods may typically involve dissolution of fluorescent dyes, such as rhodamine B, fluorescein and FPR-749, to anti-solvent water (anti-solvent) followed by addition of drug solution in organic solvents as seen in precipitation-ultrasonication method (Lu et al. 2015). Amiri et al. developed polyethylenegylcol fumarate (PEGF)-coated superparamagnetic iron oxide nanoparticles (SPIONS) for theranostic applications with good contrast comparable to that of Endorem® (It is an MRI contrast medium containing aqueous suspension of superparamagnetic iron oxide with dextran for IV administration). The authors successfully loaded tamoxifen citrate and doxorubicin into nanocrystals and evaluated the biocompatibility of PEGF-coated SPIONS (Amiri et al. 2011). Hollis et al. prepared hybrid nanocrystals of paclitaxel using anti-solvent method incorporating two flourophores, MMPSense® 750 FAST and Flamma Fluor® FPR-648. The developed nanocrystals have shown effects similar to that of paclitaxel solution along with bioimaging (Hollis et al. 2014). Poulose et al. recently developed Cu_2S based nanocrystals for trimodal imaging and photothermal therapy. Cu_2S nanocrystals were prepared by reactive crystallization at high temperatures followed by coating with lipid-polymer conjugates. Synergistic effects were observed along with multimodal imaging by photoluminescence of Cu_2S, folate targeting and chemotherapeutic effects of doxorubicin. Photoexcitation at 488 nm helped in drug release from nanocrystal-drug conjugate from the treated cancer cells within 10 min of exposure (Poulose et al. 2015). Amphiphilic plasmonic nanocrystals are composed of soft shell of amphiphilic polymers grafted on to hard metallic core nanocrystals. The hydrophobic shell and the hydrophilic aqueous cavity help in loading of therapeutic agents and diagnostic aids (photo sensitizers, florescent proteins, etc.) which in turn help in stimuli responsive delivery and synergistic therapeutic effects. Loading of photo sensitizers will help in concurrent photothermal and photodynamic therapy. In addition, excellent surface-enhanced Raman scattering and photo acoustic imaging of plasmonic vesicles helps in sensitive detection of cancer cells if they are appropriately targeted to cancer cells. (Song et al. 2015). These evolving classes of drug and metallic nanocrystal conjugates generate tremendous opportunities in guided chemotherapy and for site specific controlled drug delivery with imaging capabilities.

7.4 Safety and Efficacy

Ever increasing awareness of nanotechnology and its implications on human body and environment has lead to serious rethinking about their safety and efficacy. The parameters that determine tolerability and potential toxicity of nanosystems should be carefully considered. Mainly, size and biodegradability are two parameters that determine interactions of this system with cells and hence their fate inside the

biological system should be systematically evaluated. While looking at the "size" parameter, one thing is clear that the benefits like improved saturation solubility and dissolution by developing nanocrystals or any other nanosystems is mainly attributed to their size which is less than 1000 nm. Particles in size in range of 100–1000 nm can be taken up by cell through phagocytosis. Hence, these particles can be taken up by macrophages that are present in limited number and can be considered safer. However, particles whose size is less than 100 nm can be internalized through endocytosis by any cell. This indicates that particles below 100 nm possess higher toxicity risk as large amount of cells get exposed to these particles. Hence, particle size has been considered as a major factor while devising the nanotoxicological classification. Another important parameter is biodegradability; particles that degrade and are eliminated from the body were found to be less toxic as compared to non-biodegradable particles. This suggests the need for inclusion of biodegradability as criteria for nanotoxicological classification system.

Nanotoxicological classification as represented in Fig. 4 contains four classes after considering size and biodegradability as important parameters regarding safety of nanosystems. These classes are defined based on increasing toxicity/risk. Green patterns as depicted in Fig. 4 indicate low risk, yellow indicate medium and red signifies higher risk. Class I possess less risk as particles size is in the range of 100–1000 nm and are biodegradable in nature. When we move from class I to Class II persistency increases, means particle size is same as that of class I but these systems are non-biodegradable. However, class III nanosystems are biodegradable but particle size is less than 100 nm. Both these classes (class II and III) as represented in yellow pose medium risk. Class IV particles are non-biodegradable nature with size below 100 nm indicating that it belongs to a red colored nanotoxicological class with highest toxicity (Keck and Muller 2013). Safety is one of the prime concerns associated with medicines, thus toxicity studies are part of the most important data to be submitted for registration of new therapeutics. Safety might be a more critical aspect when dealing with the poorly soluble drugs. Large amount of

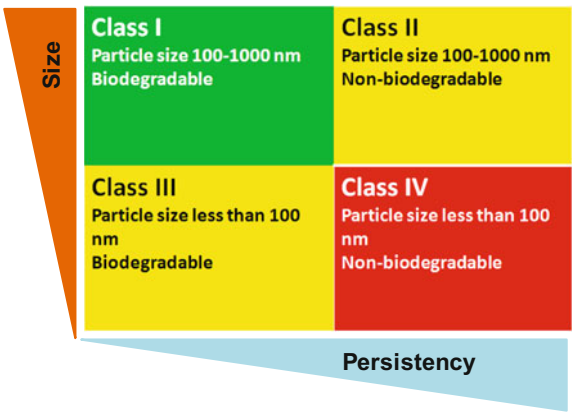

Fig. 4 Pictorial representation of Nanotoxicological classification system

solubilizers and organic cosolvents added to enhance solubility of drugs may lead to various undesired effects like hypersensitivity, nephrotoxicity, and neurotoxicity as seen with Cremophor-EL in Taxol® (Rowinsky et al. 1993; Kim et al. 2001) and renal injury with injectable formulations of itraconazole due to high amount of cyclodextrins (Rabinow et al. 2007).

8 Market Status

Nanocrystal technology competes with other advanced technologies and traditional approaches for formulating drug candidates with poor developability, since it can be readily performed in-house. They remain the most successful of all nanotechnology enabled products for drug delivery. Gris-PEG® developed using the co-precipitation was the first marketed nanocrystal product. Significant changes in the regulatory framework of drug nanocrystals are expected with the ongoing discussions revolving around quality, efficacy and safety of the nanotechnology-based products. As mentioned before, nanocrystal suspensions are stabilized by adsorption of stabilizers to the particle surface. Stabilization mechanisms and role of stabilizers used are to be clearly understood as EMA reflection paper addresses concerns related to variation in opsonization patterns due to engineered surfaces (Ehmann et al. 2013; EMA 2013). Drug nanocrystals had an estimated market size of 596 million USD by 2010 accounting for 44% of the total nanotechnology-based drug delivery market of 1.3 billion USD. Nanocrystals market is projected to increase to 60% of all nanotechnology-based products with a market capture of 82 billion USD by 2021. Lack of experience and sophisticated manufacturing facilities for scale up nanocrystal preparation has been one of the major bottlenecks for limited number of marketed products despite a convenient regulatory framework. Recent surge in marketed products and greater market capture amongst all nanoparticulate systems emphasizes the need for further development of nanocrystals (Borchard 2015). Drug nanocrystals which are currently marketed and further in development are enlisted in Table 4.

9 Concluding Remarks

Nanocrystal technology offers an efficient platform to formulate poorly soluble drugs and provide better dissolution properties with enhanced oral bioavailability. With increase in number of NCEs posing dissolution and bioavailability issues, nanocrystal technology is expected to play a significant role in drug delivery market in coming years. Simplified processes, minimal utilization of excipients, potential for large-scale manufacturing and biopharmaceutical advantages of end products makes them an ideal strategy to deal with various poorly soluble actives especially “brick dust drugs”. Nanocrsytals are versatile and can be successfully formulated

Table 4 Products of drug nanocrystals which are marketed and in clinical trials (Lu et al. 2015)

Drug	Therapy	Product	Company	Route	Technology	Method	Year
Griseofulvin	Antifungal	Gris-Peg®	Novartis	Oral	Bottom–up	Co-precipitation	1982
Verapamil	Anti-arrhythmia	Verelan PM®	Schwarz Pharma		Top-down	Media milling	1998
Sirolimus	Immunosuppressant	Rapamune®	Wyeth				2000
Dexmethylphenidate hydrochloride	Anti-psychotic	Focalin XR®	Novartis				2001
Morphine sulfate	Anti-chronic pain	Avinza®	King Pharm				2002
Methylphenidate hydrochloride	Anti-psychotic	Ritalin LA®	Novartis				2002
Diltiazem	Anti-angina	Herbesser®	Mitsubishi Tanabe Pharma				2002
Tizanidine hydrochloride	Muscle relaxant	Zanaflex™	Acorda				2002
Aprepitant	Anti-emetic	Emend®	Merck				2003
Fenofibrate	Anti-hypercholesterolemia	Tricor®	Abbott				2004
Nabilone	Anti-emetic	Cesamet®	Lilly		Bottom–up	Co-precipitation	2005
Megestrol acetate	Appetite stimulant	Megace® ES	Par Pharma		Top-down	Media milling	2005
Fenofibrate	Anti-hypercholesterolemia	Triglide®	Skye Pharma			High-pressure homogenization	2005
Naproxen sodium	Anti-inflammation	Naprelan®	Wyeth		Top-down	Media milling	2006
Theophylline	Bronchodilator	Theodur®	Mitsubishi Tanabe Pharma				2008
Paliperidone palmitate	Anti-depressant	Invega Sustenna	Johnson & Johnson	IM		High-pressure homogenization	2009
2-Methoxyestradiol	Anticancer	Panzem®	EntreMed	Oral		Media milling	Phase II
Guanylhydrazone	Anti-inflammation	Semapimod®	Ferring	IV			
Paclitaxel	Anticancer	Paxceed®	Angiotech Pharmaceuticals	IV	Unknown		
Thymectacin		Theralux®	Celmed BioSciences	–	Top-down	Media milling	
Silver	Antibacterial	Nucryst®	Nucryst Pharmaceuticals	Oral	Bottom-up	Reactive magnetron sputtering	

for drug delivery using oral, pulmonary, parenteral, ocular and topical routes, etc. Despite all the advantages of nanocrystal technology, it may not be suitable to tailor biopharmaceutical aspects of all the poorly soluble drugs. Nanocrystal may not offer an efficient solution with drug molecules which are rapidly metabolized and display poor permeation properties. Moreover, issues related with intercellular uptake, role of stabilizers with P-gp inhibitory effects in bioavailability enhancement, stability concerns due to phase transformations during solidification process are inadequately addressed to date. Looking at growing number of marketed products of drug nanocrystals one would be optimistic to foresee a very bright future in the field of nanocrystal technology.

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