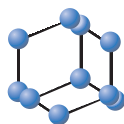
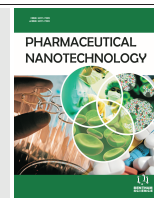


## REVIEW ARTICLE


**BENTHAM  
SCIENCE**

# Nanocrystallization: An Emerging Technology to Enhance the Bioavailability of Poorly Soluble Drugs


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**Abstract:** Most of the active pharmaceutical ingredient used in the management of disease have poor water solubility and offer grueling problems in drug formulation development since low solubility is generally associated with poor dissolution characteristics which leads to poor oral bioavailability. The great challenge for the development of a pharmaceutical product is to create its new formulation and drug delivery system to limit solubility problems of existing drug candidate. Limited drug-loading capacity requires a large amount of carrier material to get appropriate encapsulation of the drug, which is another major challenge in the development of pharmaceutical product which could be resolved by developing nanocrystals (NCs). A significant research in the past few years has been done to develop NCs which helps in the delivery of poorly water soluble drugs *via* different routes. The technology could continue to thrive as a useful tool in pharmaceutical sciences for the improvement of drug solubility, absorption and bioavailability. Many crystalline compounds have pulled in incredible consideration much of the time, due to their ability to show good physical and chemical properties when contrasted with their amorphous counterparts. Nanocrystals have been proven to show atypical properties compared to the bulk. This review article explores the principles of the important nanocrystallization techniques including NCs characterization and its application.

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## 1. INTRODUCTION

Research in different pharmaceutical drug formulation encounters remarkable problems related to poorly soluble compounds [1]. Drugs which have low water solubility leads to poor bioavailability which in turn creates major problems in the development of formulation resulting in significant delays in the pharmaceutical screening of drug [2]. Techniques like salt formation, complexation,

solubilization, pH alteration, cosolvents, surfactant, microemulsions, self-emulsifying drug delivery system (SEDDS), or self-micro emulsifying drug delivery system (SMEDDS), micronization of drug, chemical modification [3-5], nanovesicles and NCs delivery system have been investigated and used widely to increase the solubility of drug but they all are not universal solution because of some limitations like drug loading, scale-up difficulties, quality control difficulties, adverse effect of excipient on stability of drug chemically and pharmacologically [6-8]. Conventional techniques used for poorly water-dissolvable medications includes solvent blends, cyclodextrines, solid dispersion and o/w emulsions. But every one of these methodologies are constrained because they require

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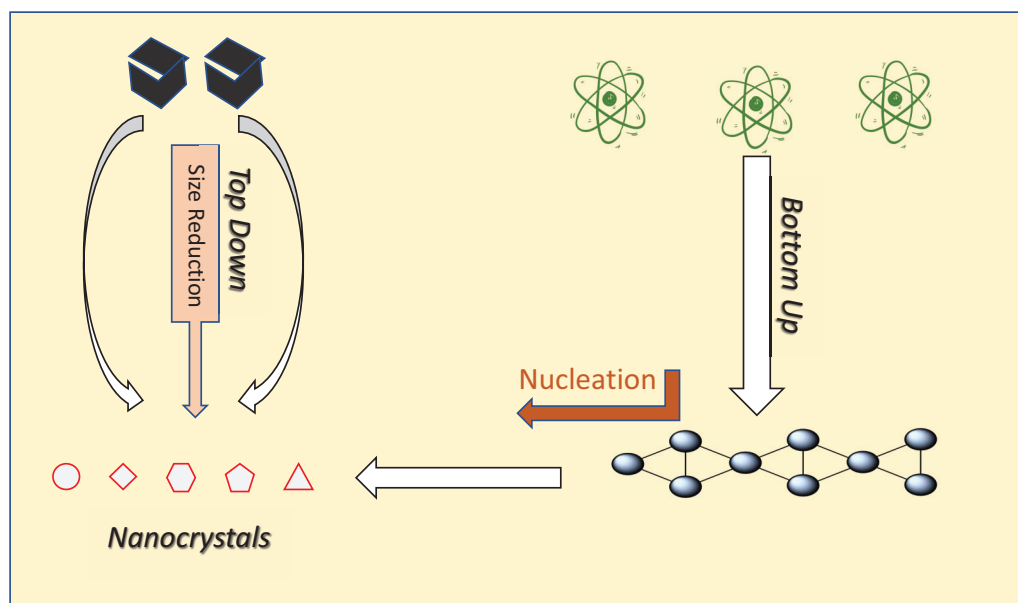
molecule with specific properties. Furthermore, patients would be presented to solvent blends with some additional solubilizing agents, prompting side effect and lethal responses. To minimize the use of excipients which increases the solubility and bioavailability of drug, examination of innovative methodologies is an earnest need and nanocrystallization has risen as an important tool to improve their pharmacokinetics [9]. The use of reducing the particle size approach to make uniformly sized formulation in nanometer range has been exposed to be quite an advantageous formulation strategy [10]. The NCs provide excellent novel functions by controlling the crystal growth, morphology, size, crystallinity, anisotropy, orientation, self-assembly, integration, *etc.* Each crystal has a different crystal structure, Amorphous or crystalline. NCs are crystalline bunches of drugs which are carrier free [11] with no excipient material with size measure extending from 10 to 1000 nm. NCs not only enhance dissolution rate velocity, it also improves saturation solubility as well and hence, a higher bioavailability can be logically achieved due to their high surface to volume ratio, which ultimately increases both the dissolution rate and saturation solubility of active pharmaceutical ingredients [12]. The NCs could be easily formulated into conventional dosage forms for oral, pulmonary, nasal, ocular and injectable administrations [13, 14]. Many different techniques for nanocrystallization to form NCs like precipitation, spray drying, milling, high speed homogenization *etc.* have been explored by scientist [15]. In comparison to the matrix and encapsulated type particles in which drug is distributed evenly on the surface and present in the center/core, respectively, which make total drug less than 100% available always but nanocrystal consist of 100% drug which makes them highly efficient in transporting the drug into the cells, reaching a sufficiently high therapeutic concentration for the pharmacological effect [16]. The Nano crystallization techniques are mainly divided into bottom-up, top-down and (mixed) combinatorial approaches [17]. In top down (destructive) approaches particle size is reduced to Nano range by attrition or milling and in bottom up (Constructive) techniques particle in Nano range is formed using nucleation or crystal growth approaches. Combination techniques contain mix of both (bottom-up and top-down) approaches [18].

## 2. NANOCRYSTAL

Drug NCs are nanoscopic crystals of parent compounds with a dimension of less than 1 $\mu$ m. NCs are Nano-range particles composed of 100% drug without any matrix material. Poorly soluble drugs encounter biopharmaceutical delivery problems such as low bioavailability after oral administration, low penetration of the drug into the skin, large injection volume for intravenous (I.V.) administration and undesired side effects after I.V. injection when using traditional formulations. The unadulterated drug crystals might be physically stabilized by surfactants or polymers [19]. Elan Pharma International Ltd. (Ireland) registered a trademark named NanoCrystal<sup>®</sup> that offers a skill which help in improving the bioavailability of drugs by reducing them as nanosized particles that can be made into powder or suspended in liquid and formed as tablet or can be encapsulated in the same [20, 21]. Above all, physical instability issues inherent with different nanocarriers are to a great extent avoided by the NCs plan or approach [22, 23].

NCs are generally formulated as Aq. dispersion (nanosuspensions), which need further solvent elimination processes to get re-dispersible powders which showed up very quick availability of formulation in market from the year 2000 onwards. Despite one exemption, all available products in market till now are of oral delivery; they are all solid dosage forms (tablets or capsules), but only one preparation is a suspension called Megace ES [24].

Chemically labile drugs can be stabilized by using the technique of nanocrystallization to form their nanocrystal. The drug paclitaxel has been formulated as a nanosuspension for its preservation from degradation [25]. Today, it is possible to prepare and study NCs of metals, semiconductors and other substances by various means. NCs of materials are generally obtainable as solutions. Sols containing NCs behave like the classical colloids. NCs dispersed in liquids are either charge-stabilized or sterically stabilized. Nanocrystal suspensions were transformed into dry powders by applying both granulating techniques and freeze-drying. The dry powders can be further processed to form tablet and capsule formulations which can



**Fig (1).** Two approaches for Nanocrystal preparation.

be tested *in vitro* and *in vivo*, and they have shown great promise in terms of biomedical applications, ranging from novel drug delivery platforms and drug targeting to diagnostics and tissue engineering [26].

Limitations of NCs are given below:

1. High energy required for nanonization of drug size.
2. Requirement of stabilizer.
3. Not for low therapeutic indices drug.
4. Limited control over release [27].

## 2.1. Methods of Preparation

Predominantly two essential methodologies are engaged with the preparation of NCs the top-down technologies (Reducing to nano size of the large-size drug powder *e.g.* by mechanical attrition or wear down) and the bottom-up technologies (controlled precipitation /crystallization) (Fig. 1).

However, the combination techniques, consolidating a pre-treatment with a subsequent size reduction step are additionally being utilized [28]. This review focuses on the various production technologies available till now, also *e.g.* supercritical fluid technologies and solvent evaporation [29].

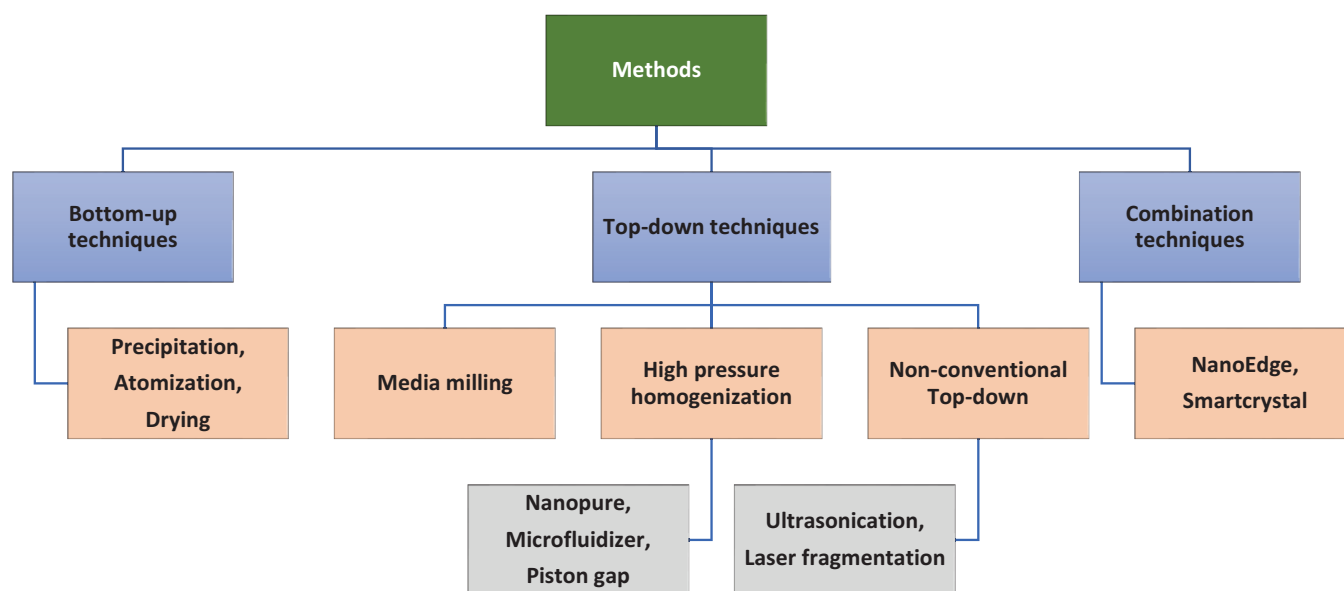
High-pressure homogenization, milling and precipitation are the key strategies utilized to prepare drug NCs. The significance for enhancing bioavailability of the poorly-soluble drugs by the production of drug NCs is generally acknowledged at this point [30]. The top down methods basically include homogenization or milling while the bottom-up methods are primarily based on the principle of precipitation. The combination approaches include both top down method plus bottom down method. The main production technologies currently in use to produce drug NCs yield as a product a dispersion of drug NCs in a liquid, typically water (so called “nanosuspension”). Classification of nanocrystalization techniques is shown in Fig. (2) and some of the aspects of two approaches are classified in Fig. (3).

### 2.1.1. Top Down Approaches

High-energy mechanical forces are associated with the top-down approaches, which can be given by either high- pressure homogenization (HPH) (IDD-P<sup>®</sup>, DissoCubes<sup>®</sup> and Nanopure<sup>®</sup>) or media milling (MM) (NCs<sup>®</sup>) to pulverize large crystals [17, 31].

#### 2.1.1.1. Media Milling (NCs<sup>®</sup>)

Nanocrystalline dispersions are formulated by using media milling processes [32]. A milling chamber, recirculating chamber, motor, milling



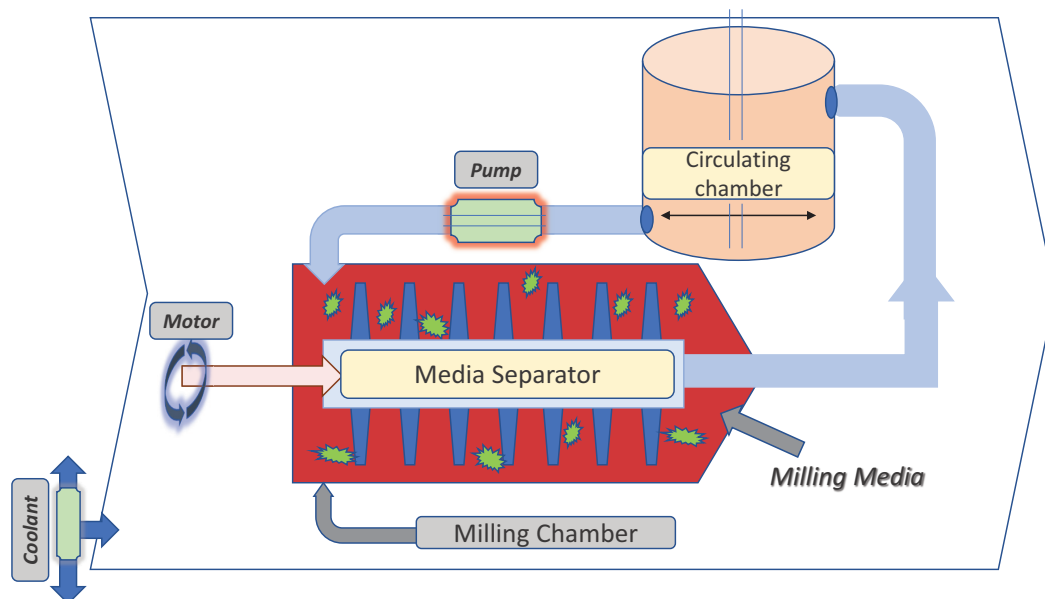
**Fig (2).** Preparation techniques of Nanocrystal.

TOP DOWN		BOTTOM UP
Start with Larger crystal and break them down mechanically	<b>Approach</b>	Start with drug molecule in solution which form nanocrystal in changed condition
<ul style="list-style-type: none"> <li>- Industrial capable</li> <li>- Easier to control the size</li> <li>- Avoid the use of organic crystal</li> </ul>	<b>Advantages</b>	<ul style="list-style-type: none"> <li>- Low energy input</li> <li>- Use for thermolabile drugs</li> <li>- Possible to prepare small particle</li> </ul>
<ul style="list-style-type: none"> <li>- More expensive and high energy input</li> <li>- Long processing time</li> <li>- Contamination from the Equipment</li> </ul>	<b>Disadvantages</b>	<ul style="list-style-type: none"> <li>- Crystal growth need to control</li> <li>- Require good solvent</li> <li>- Remove toxic organic solvent</li> </ul>
Wet ball milling and high pressure homogenization	<b>Example</b>	Supercritical fluid technology, Precipitation

**Fig (3).** Different aspects of Nanocrystal.

media and coolant are the key components of the media mill. The processing chamber is filled with a raw slurry of drug, stabilizers and water. In the process, the milling chamber is fed with a crude slurry of drug, water and stabilizers, and this was agitated by the motor. By the end, the slurry occupies 2%-30% (w/v) space in the milling chamber, while the milling media takes 10%-50% (w/v) of the slurry [33]. The reduction in size is achieved by the mechanical attrition and shear that developed due to the impact between a drug particle and the dividers of the processing chamber or processing media and drug particle or additionally

between the two drug particles. The processing media are pearls or little globules made of ceramic (e.g. yttrium stabilized zirconium dioxide) or very cross-connected polystyrene resin or stainless steel or glass having diverse sizes (0.3 mm or higher). However, the need is to prevent the formulation from contamination. Media milling technology is shown in Fig. (4). Range of concentrated drug is from 1 to 400 mg/ml. Increased power created shear powers and/or the powers produced during impaction of the processing media with the medication give adequate vitality contribution to break drug crystal into nanometer measured particles.



**Fig (4).** Media milling equipment.

Recirculation is very advantageous to reduce milling time and decrease the particle size. The media milling process readily breaks micron-sized drug crystals into the dispersion of homogeneous nanoparticles. Therefore, the coolant is necessary to control the temperature during the size reduction process [17, 18, 34, 35].

#### 2.1.1.1.1. Process Variables in Nanomilling

Parameters to acquire ideal product through nanomilling, have been found to incorporate and influenced by different factors like specific amount of drug, number of the processing pearls and size, milling time, processing speed and temperature. Ordinarily, the amount of medication in the chamber is very low, from 2 to 30% (wt.), while the number/volume of the milling pearls/beads is high, 10-50% of the volume of the slurry. The size of the nanomilling pearls is kept consistent somewhere in the range of 0.5 and 1.0 mm. The handling times and speeds required to get NCs of appropriate size range vary significantly. NCs are produced either by low milling velocities (80-90 rpm) and longer processing time (1-5 days) or high milling speed (1800-4800 rpm) and shorter processing times (30-60 min).

#### 2.1.1.2. High Pressure Homogenization (HPH)

HPH is another top down procedure where the drug particle size is get reduced by shear powers, cavitation powers and drug particle colloid which

is supported by high pressure conditions. There are of two type which were known by the micro-fluidization and piston gap homogenization.

While in HPH processing, drug suspensions are brought into a high-pressure homogenizer and went through a restricted homogenization pathway in an abrupt burst under high pressure:

1. **Micro-fluidization** is also called jet stream homogenization or air-jet milling wherein the particles are fragmented in a high pressure air jet induced by the collision of two fluid streams under the pressure of 1700 bar [36].
2. **Piston-gap homogenization** utilizes high pressure to constrain a fluid suspension through a narrow channel or a little gap inside a pipe. This procedure is commonly made out of three stages: (1) Drug powder dispersed in a pure solution or in a solution containing stabilizer, (2) Molecule size reduction by rapid shearing or low-pressure homogenization and (3) Use of high-pressure homogenization to get the reasonable particle size and size distribution.

#### 2.1.1.2.1. High Pressure Homogenization in Water (Dissocubes)

Trade name of the prepared nanosuspensions is Dissocubes<sup>®</sup> as the particles have extraordinary

dissolution/disintegration properties and a cuboids shape. This innovation does not cause the erosion of handled material and adulterated from the production/processing equipment and that should be ordinarily below from 1 ppm that implies within an appropriate range. Exceptionally concentrated as well as diluted nanosuspensions can be prepared up by handling of 1mg/ml to 400mg/ml amount of drug, relevant to the drug that are have low solubility in both watery and non-Aq media and permits aseptic production of nanosuspensions for parenteral administration [37].

#### 2.1.1.2.2. Homogenization in Water Free Media and Water Mixtures (Nanopure)

For some delivery routes, it is more suitable to have drug NCs spread in non-aqueous media. When developing the second generation of drug NCs (Nanopure), drug suspensions in non-aqueous media such as propylene glycol were homogenized [38, 39].

#### 2.1.1.2.3. HPH Process Variables

To get an improved formulation from the homogenization technique, the following procedure parameters which have some impact on properties of NCs must be considered such as:

- Pressure that applied
- No of times Homogenization cycles runs
- Temperature

Usually, the homogenizer can handle varying pressures, ranging from 100 to 1500 bar for most lab-scale ones. Therefore, an effect of homogenization pressure on the particle size should be investigated to optimize the final formulation. The increased cycle numbers provide more energy to break down the crystals. Therefore, homogenization is often performed in five, ten, or more cycles depending on the hardness of drug and the desired particle size. Raising the temperature in the homogenization process is not favorable to thermo-sensitive drugs. In that case, the temperature can be easily reduced by placing a heat exchanger before the homogenizer valve. In general, temperature of the sample can be maintained at about 10°C.

The biggest advantage in the top-down process is that it is a universal technique to prepare crystal-

line nanoparticles and is flexible in production scale [40]. Thus, the process has been widely adopted to prepare commercial NCs. Almost all commercial products were produced by media milling except for Triglide® by homogenization®. It allows aseptic production of nanosuspensions for parenteral administration and it is universally applicable and easy down-stream processing.

Disadvantages of the top down process are as follows:

- This technology utilizes high amount of energy and more time consumption as well as adulteration from the grinding media.
- The milling time varies from hours to days, depending on the properties of the drug, the milling media, and the extent of particle size reduction [18, 41].
- The top-down process may not be the good alternative to prepare NCs for I.V. injection.

#### 2.1.1.3. Non-Conventional Top-Down Method

##### 2.1.1.3.1. Ultrasonication

This involves reduction of particle size by high intensity in a liquid nitrogen suspension. During the sonication process, the drug is suspended in liquid nitrogen to prevent over-heating of the sample. Generally polyelectrolyte's, such as polycations (polyallylamine hydrochloride and polydimethyldiallylamide ammonium chloride) and polyanions (poly-styrene sulfonate) [42] are incorporated in the sonication medium. Primary disintegration, the polyelectrolytes cover the surface of the nanoparticles due to electrostatic interactions also gave stability to agglomeration during charge repulsion or modification of the zeta-potential, but can also affect the dissolution properties of the particles (as the number of coating layers increase). The opportunity of controlling the release rate of drug is depend on the particular polymer coat used [43].

##### 2.1.1.3.2. Laser Fragmentation

Particle size reduction by laser fragmentation has recently been applied to pharmaceutical systems [44]. NCs can be obtained through ablation followed by fragmentation. In this a laser beam is

directed towards microcrystals embedded in a static liquid resulting in the dispersion of the solid into the liquid. During the fragmentation step, the coarse suspension is stirred and irradiated obtaining a nanosuspension. Mainly API degradation, happen when the laser power is increased [45].

### **2.1.2. Bottom-up Techniques**

The bottom-up process forms NCs from solution, which comprises two main steps *i.e.* consequent crystal growth through nucleation process. In contrast to achieve small and uniform NCs, nucleation pathways are much more adaptive and used. Firstly, the molecules are present in solution phase, then the molecules are aggregated/combined to form particles which can be amorphous form or crystalline form. Nucleation can be triggered either by mixing with antisolvent or removal of solvent. This method can be named as ‘a classical precipitation process’. In this method, the drug is totally dissolved in a suitable solvent. Then this solvent solution is added to a non-solvent, causing precipitation of the drug. The mixing of drug solution and nonsolvent can be attained with conventional mixing equipment, *i.e.* agitator blade and magnetic stirring. To promote nucleation of particles, sonication can be used to provide cavitation effects. This method is called sonoprecipitation or sonocrystallization. However, an essential drawback of these precipitation method is that the utilization of a natural solvent which should be removed, prompting the high cost production. Precipitation has likewise been utilized in combination with homogenization. The significant limitation with precipitation is the uncontrolled development of particles which has resulted in its acceptance for only a few selected molecules. Especially, if there should arise an occurrence of low Aq and non-Aq dissolvable drug, the high amount of solvent volumes is required. Consequently, in the pharmaceutical business, the bottom up procedures have not been utilized to deliver the marketed drug. One exceptionally effective blending equipment has additionally been utilized to deliver NCs, including the confined impinging jet reactor, static mixer and multiple inlet vortex mixer. With these instruments, exceptional micro mixing scale blending between the two liquids would be possible in milliseconds. Recently, spray-freezing into fluid and controlled crystallization during freeze-

drying have additionally been developed to prepare NCs by evacuation of the solvent [46].

Process variable of precipitation: The outcome of the process variables, temperature, rate of stirring and infusion rate of solute solution into the antisolvent, was streamlined in terms of how these aspects influence the local supersaturation achieved at the initial phases of precipitation. The influence of processing parameters (volume and temperature of the heated aqueous solution; type of nozzle) and formulation aspects (total solid concentrations; stabilizer concentrations) on the size of suspended particles, can be determined by laser light diffraction. But, the size distribution of dispersed nanoparticles was shown to be mostly independent across the different formulation and processing parameters [47, 48].

It was studied that the kind of stabilizer used in the heated aqueous receiving solution is much more important than its actual temperature as an increase in temperature either resulted in an increase or decrease in the mean particle size. Processing also does not require a customized atomizing nozzle to produce submicron or micron-sized particles.

Supercritical fluids (SCF) can also be used to make NCs by taking benefit of the exclusive physical characteristics of SCF, with joint diffusivity like gas and solubilization like liquid. Additionally, easy and fast elimination of SCF without too much drying can significantly facilitate the precipitation of nanoparticles [49]. A problem related with this method/technology is that the created nanoparticle is required to be stabilized to avoid growth in micro to metre crystals. Lyophilization is sometimes suggested to attain the predefined particle size. One more approach is to reserve the size of the precipitated NCs, by using polymeric growth inhibitors, if water phase viscosity increases then it can decrease particle growing.

#### **2.1.2.1. Rapid Expansion in Supercritical Solutions and Spray Flash Evaporation**

Currently, two different methods showed up: The Rapid Expansion in Supercritical Solutions (RESS) and the Spray Flash Evaporation (SFE). Concerning RESS, the procedure can create nano-organic materials, for example, explosives or nano

size drugs by crystallizing them in a reasonable supercritical dissolvable, for example, high pressure carbon dioxide and high temperature. This is accomplished in a continuous way [50]. Even though these strategies can produce constantly well dried formulation, because of the low solvency of the materials in carbon dioxide, the production amount limit is still low, barely achieving production limits more than 1 gram/hour. In order to avoid each of these restrictions of various techniques, the Spray Flash method was created.

The Spray Flash Evaporation (SFE) system was planned by the ultra sound splash pyrolysis method. The compound to be nanocrystallized, an undiluted one or a blend of mixes, is broken down in a low boiling organic/natural solvent for example ethanol and acetone, in these solvents, the dissolvability of each compound is for the most part involved somewhere in the range of 1 and 10 wt. % at room temperatures. At that point the arrangement is pressurized to ~ 40bars, by compressed nitrogen gas which extended through a warmed empty cone nozzle into a primary vacuum chamber. The nozzle is warmed at temperatures in between 130 and 160°C, while the evaporation chamber is set under vacuum at 520mbar pressure. The extraordinary pressure drops from the high-pressure zone to the low-pressure zone with ensuing temperature drop initiate evaporation instantaneously and consequent crystallization of the particles that was dissolved in the original solution. After its crystallization, the item can be trapped in a cyclone, in an electrical precipitator or in filter [50].

### **2.1.2.2. Stress-Induced Seed-Assisted Nanocrystallization**

Recently, novel platforms using bottom-up nanocrystallization method that is commonly applicable to numerous compounds in spite of the differences in their chemical structures and size.

The process is designed to intensify the rate of crystal growth by nucleation and reduce their growth rate in three phases: Seeding, ultrasonication and precipitation. In the final precipitation phase, a highly concentrated solution of the compound is injected into a high volume of antisolvent, that's leads to form amorphous nanoaggregates. Drug crystal seed are then incorporated to

the suspension and the least amount of the solvent is added to give the least freedom to molecules that are mandatory for crystallization. To avoid the unnecessary growth of branches on the seeds, and to quicken their separation, mechanical stress, like ultrasonic waves, is introduced to the suspension. The suspension is sonicated for a couple of minutes to build its dissolution and crystal nucleation on the seeds and detachment of crystal from the seeds [51, 52].

### **2.1.3. Combination Technology (NanoEdge<sup>®</sup>, SmartCrystal)**

There is thorough research continuing for new innovations prompting numerous different ways to deal with produced drug NCs. The combination technique consolidates commonly a pre-treatment step pursued by a high energy step, for example, the NanoEdge<sup>™</sup> innovation. In the pre-treatment step, crystals are precipitated; and the resulted suspension is then exposed to a high energy process, ordinarily a high-pressure homogenization. As indicated by the patent cases by Baxter, the annealing step stops the development of the accelerated NCs. Annealing is characterized in this advancement as the act of changing material that is thermodynamically unstable into an improved thermodynamically stable structure by single or constant utilization of energy (mechanical pressure or direct heat), followed by a thermal relaxation step.

The SmartCrystal<sup>®</sup> skill is developed and owned by Abbott and marketed by its drug delivery company SOLIQS in Ludwigshafen/Germany. SmartCrystal innovation is not just a single innovation however has a wide range of procedures that are consolidated either to quicken production by reducing the number of passes through the homogenizer or to acquire little NCs of size underneath 100 nm. Such small sized NCs are not easy to produce through pearl milling or normal high-pressure homogenization, particularly in large scale mechanical preparation [53]. The mix procedure H69 is a parallel stream precipitation and ensuing high-pressure homogenization (HPH) in which the precipitation is shaped in the cavitation zone or just before the cavitation zone of the homogenizer (cavi-precipitation). In the H42 procedure, high-pressure homogenization and spray dry-



ing is consolidated. Additionally, in H96 process, the high-weight homogenization (top down) and lyophilization (bottom up) are joined to yield NCs which is most effective combination technology. Processes H69 and H96 is the ability to produce crystals below 100 nm, a range practically not accessible by high pressure homogenization alone. Spray drying or lyophilization of the drug solution leads to a powder more susceptible to be broken in the subsequent high-pressure homogenization step. The SmartCrystal technology is considered as the second generation of drug NCs [54].

### 3. STABILIZING AGENTS FOR NCS AND THEIR EFFECT ON BIOAVAILABILITY

Distribution of drug NCs in liquid media leads to so called “nanosuspensions” (in contrast to “microsuspensions” or “macrosuspensions”). In general, the dispersed particles need to be stabilized, such as by surfactants or polymeric stabilizers. Dispersion media can be water, aqueous solutions or non-aqueous media. For most nanocrystalline formulations, drug concentration is 400 mg / ml or less. The choice and concentration of stabilizer are selected to promote the particle size reduction process and generate physically stable formulations. To be effective, the stabilizer must be able to wet the surface of the drug crystals and providing a steric or ionic barrier. In the absence of the appropriate stabilizer, the high surface energy of nanometer sized particles would tend to agglomerate or aggregate the drug crystals. Physically stable nanocrystalline formulations are obtained when the weight ratio of drug to stabilizer is 20:1 to 2:1. Too little stabilizer induces agglomeration or aggregation and too much stabilizer promotes Ostwald ripening. The process of identifying an appropriate stabilizer(s) for a drug candidate is empirical and can be accomplished using amount of drug in milligram. Pharmaceutical excipients such as the polysorbates, cellulosic, povidones and plutonic are usually used that are acceptable stabilizers for creating physically stable nanoparticle dispersions. NCs are noticeably easy to prepare, but then again the stability and the selection of stabilizer(s) greatest challenging and critical step [41]. The newly created drug NCs are stabilized by the stabilizer, but they also have a vital role in another types of formulations and they even influ-

ence bioavailability of drug in body, so before making stabilizer, it should be kept in mind. Choice of stabilizer can dramatically affect the performance of the optimized NCs during the further formulation steps and *in vivo*.

Advancement of nanosized particles makes high energy surfaces, which can spin to aggregate and Ostwald ripening, if stabilization isn't at an efficient level.

Stabilizers are surfactants, polymers or amphiphilic. Although greater part of the API materials that are promising candidate for NCs preparation are poorly/inadequately dissolvable and hydrophobic, the amphiphilic stabilizers will likewise enhance the wetting and disintegration properties of these materials [55, 56]. Stabilizers can be non-ionic or ionic in nature and the general steadiness depends on the established DLVO-hypothesis came to either by means of electrostatic powers or steric block.

- i. Polymers : HPMC, HPC, MC [57, 58], PVP [59], poloxamers [59, 60].
- ii. Nonionic: Polysorbates, Sorbitan esters, vitamin E TPGS [61, 62].
- iii. Ionic: SDS [58].

### 4. CHARACTERIZATION OF NCS

Most commonly employed characterization techniques for NCs [63-65] are given in Table 1.

NCs are mainly formed as nanosuspension as a product but, the most convenient dosage form for the patient is a dry product, *e.g.* tablet or capsule. So, formulation of tablet and capsule homogenization of NCs can be performed in polyethylene glycols being liquid at room temperature [66]. It is a solid dispersion of drug NCs in solid PEG as outer phase. In a subsequent step milling can be performed yielding a flowable powder. The powder can be admixed to a standard mixture used for direct compression. As lined out above, the liquid PEG nanosuspensions can be filled into soft gelatin capsules or alternatively into hard gelatin capsule which are subsequently being sealed. *In vivo* studies of NCs are performed according to the route of administration and thus animal model is designed.

**Table 1. Parameters for NCs evaluation.**

Characterization Parameter	Examples of Analytical Methods
1. Structure and morphology	Scanning electron microscopy, Light microscopy, transmission electron microscopy, atomic force microscopy, field emission scanning electron microscopy.
2. Surface charge	Zeta potential by Zetasizer.
3. <i>In-Vitro</i> release characteristics	USP dissolution apparatus, Stable buffer medium, pH, and temperature [60]. Fluorescence microscopy [61].
4. Rheological properties (for liquid nanosuspensions)	Viscometer, rheometer.
5. Solid state analysis (crystallinity)	Differential scanning calorimetry, Powder X-ray diffraction.
6. Particle size and particle size distribution	Laser diffraction (static laser light scattering), Photon correlation spectroscopy (based on dynamic laser light scattering), microscopic methods.

## 5. MECHANISM OVER INCREASING SOLUBILITY

Decrease in particle size to nano range *via* nanocrystallization results in improved saturation solubility as well as dissolution velocity [67, 68]. The relation between the saturation solubility of a drug and its particle size is inversely proportional to each other (according) to which decrease in particle size results in increase in Surface area consequently saturation solubility of the drug [69]. NCs offer large surface area which increase the contact area of each particle and solvent system which tremendously increase the passing of drug from bulk to solution hence dissolution velocity is fastened [70, 71]. This relationship can be expressed by simple Noyes Whitney equation,

$D$  = diffusion coefficient,

$A$  = surface area,

$C_s$  = saturation solubility,

$C_x$  = bulk concentration,

$h$  = Diffusional distance over which the concentration gradient happens

The drop of particle size, increased saturation solubility, an enlarged surface area and a thinner diffusion layer can intensely increase the dissolution velocity, which ultimately improves bioavailability of drug in body [24]. Therefore, reduction in particle size is a good approach to successfully improve the drug bioavailability where the drug's

dissolution speed is the rate limiting step [34]. *Via* moving to nanonization from micronization, the rate of dissolution increases because of particle shell is further increase. In many cases, a low dissolution speed is related to low saturation solubility [11] but by enhancing the saturation solubility concentration gradient between the blood and gut lumen, then the absorption by passive diffusion [18]. Like other nanoparticles, NCs showed up an increased or improved adhesiveness to tissue which generally lead to an enhancement in oral absorption of poorly soluble drugs apart from the increased dissolution rate and saturation solubility [72, 73]. It is well known that amorphous drugs possess a higher saturation solubility than crystalline drug material. Amorphous drug nanoparticles possess a higher saturation solubility as compared to equally sized drug NCs in the crystalline state. Therefore, to achieve the highest saturation solubility [74], a combination of nanometer size and amorphous state is ideal.

To establish, the optimal drug NCs size and crystalline state/ amorphous, it all depends on the required blood profile for drug. Administration route and Stability of the amorphous state during shelf life of the product [75].

## 6. CLINICAL APPLICATION OF DRUG NCs

### 6.1. Oral Delivery

The formulation of drug NCs can impressively improve the bioavailability of orally administered

poorly soluble drugs. Whenever a quick onset of a poorly soluble drug is desired, the formulation of drug NCs can be favorable, for example, in case of analgesics. Naproxane is an analgesic, formulated as a nanosuspension, has exposed a reduced  $t_{max}$  but concurrently approximately three fold increased AUC in comparison to a normal suspension (Naprosyn<sup>®</sup>) [20] plus a reduced gastric irritancy [76, 77]. An additional key advantage of drug NCs is their adhesiveness and the increased residence time, which can positively manipulate the bioavailability. The mucoadhesiveness can be increased by the use of mucoadhesive polymers in the distribution medium [78, 79].

## 6.2. Parenteral Administration

The parenteral application of poorly soluble drugs, particularly intravenous (IV) administration of practically insoluble compounds, using cyclodextrines, surfactants, liposomes, or cosolvents, is often associated with large injection volumes or toxic side effects. Carrier-free nanosuspensions facilitate possible higher loading capacity compared to other parenteral application systems. When a drug is administered as a nanosuspension, the rapid dissolution of the NCs will mimic the plasma concentration profile of a solution [80]. The nanosuspension was much better tolerated, resulting in an approximately doubled LD50 value [81].

## 6.3. Pulmonary Drug Delivery

Many important drugs for pulmonary delivery show poor solubility simultaneously in water and non-aqueous media, for example, some important and widely used corticosteroids such as budesonide or beclomethasone dipropionate. Nanosuspensions are capable to be effectively applied to solve these problems. The nebulization of nanosuspensions generates aerosol droplets of the desired size loaded with a large amount of drug nanocrystals [82]. Using these nebulized nanosuspensions, the respirable fraction is distinctly increased than conventional MDIs [83]. Besides this, drug NCs show an increased muco-adhesiveness, leading to a prolonged residence time at the mucosal surface of the lung.

## 6.4. The Ocular Delivery

Nanoparticles including drug NCs is also of high interest in ocular delivery. The enlargement

of such colloidal delivery systems for ophthalmic use aims at droppable dosage forms with elevated drug loading and a long-lasting drug action. The adhesiveness of the small nanoparticles, which can be further increased using mucoadhesive polymers, leads to a more consistent dosing. Blurred vision can be reduced by the use of submicron-sized drug particles [84].

## 6.5. Topical Delivery

NCs exhibit the properties like enhanced permeation, bio adhesiveness and increased penetration into membrane. However, for many years no attention was given to exploit adhesion, fast dissolution and increased penetration for dermal and mucosal application. This helps when the poorly soluble antioxidants Rutin, apigenin and Hesperidins were formulated as nanosuspension for application in skin protective, anti-aging cosmetic products. The NCs are simply admixed to the water phase of dermal creams and o/w lotions. These products contain nanosized crystals but are not a nano product according to the new European regulations for cosmetics, as the size of the NCs is above 100 nm, and the particles should not be biopersistent, they should be biodegradable. The underlying mechanism of action is: the NCs increase the solubility of the poorly soluble active in the aqueous phase, this leads to a higher concentration gradient. The identical principle can be applied to pharmaceutical dermal formulations. Diclofenac sodium nanosuspension for transdermal delivery showed higher permeability flux of drug across the skin by up to 3.8 fold as compared to the control when tested in Yucatan micropig (YMP) skin model [85, 86].

## 6.6. Target Drug Delivery

The requirement to target drugs to specific sites by means of nanoparticles is increasing day by day because of economic and therapeutic factors. Nanosuspensions are able to be used for targeted delivery as their exterior properties and *in vivo* performance can easily be changed. Pulmonary aspergillosis can be easily targeted by employing amphotericin B nanosuspensions instead of using stealth liposomes. Important for IV targeting is the modification of the surface properties of the NCs. The surface properties of the NCs determine the

qualitative and quantitative composition of the adsorption patterns of blood proteins. The surface properties can be adjusted in such a way, that the particles even adsorb automatically to the blood proteins liable for the enrichment at the desired target site [87].

## 6.7. Miscellaneous

### 6.7.1. Semiconductor NCs in Health

Nucleic acids, enzymes and membranes and so on are the elementary practical units comprised of complex nanoscale particles in biological frameworks. In past, scaling down where metals, semiconductors and magnets through which we build optical and electrical sensors, would now be able to be set up on the size of individual biological macromolecules that will have significant effect on anticipated medicinal treatments. In delivery system and clinical applications, nanotechnology is one of the key variables for present day/modern treatment. Attributable to the straightforwardness in the preparation and common utility NCs which are free of novel carrier free colloidal delivery system having a size of particle ranges 100-1000 nm, these NCs is an efficient drug delivery approach to build up improved soluble preparation from poorly soluble drug. Research has being made on the colloidal quantum dots because of their steady light emitting nature, which can be extensively tuned by fluctuating the size of the NC. Loads of research work have been done in most recent two years on the improvement of huge scope of techniques for bio conjugating colloidal NCs [88] as a result of their differing applications, for example, *in vivo* imaging [89], cell tracking [90], DNA detection [91] and so forth. Although the truth that studying the visual properties of NCs, it has been seen that NCs fluorescence wavelength emphatically relies upon their size as a result of which NCs photo bleach property get diminished. These NCs can be used in fluorescence tests for different kinds of labeling examination like tracking the path and absorption of NCs by living cells and cellular structures labeling [92].

### 6.7.2. NCs in Nutritional Health

The nutraceutical market is growing, and there are many nutraceutical compounds, *e.g.* antioxidants, which are poorly soluble. It was shocking

that in some marketed available products *e.g.* hesperidin, the amount of drug dissolved after half an hour was still nearby to zero. NCs can be capably used to deliver effective, bioavailable nutraceutical products in future.

## 7. MARKETED NCs

Conventional treatment and some novel carrier system have the disadvantage of stability efficacy, bioavailability and toxicity; therefore, some of the drugs are formulated in the NCs form and commercialized for effective treatment. Some of the Marketed NCs and their method of preparation are summarized in Table 2 [93-108].

## 8. NANOTOXICOLOGY OF NCs

In the last 2-3 years there is an increasing concern about potential nanotoxicity of nanosized particles, since when we move to the nano size range, change in physicochemical properties can also give them also potentially new toxic features. Therefore, nanotoxicology is getting an increasingly important role, while developing safe nanocarrier [96]. Particles of major toxicological concern are the particles below 100 nm (*e.g.* FDA, European Cosmetic Regulation (Kislalioglu, 1996). The background is that properties of particles <100 nm are again very much different to large nanometer particles (*e.g.* 200-800 nm). Example: Large nanometer particles can only be internalized by macrophages (limited cell number in the body), and cause effects inside the cell. Particles below about 150 nm can be internalized by any cell of the body *via* pinocytosis. That means these particles can access any cell of the body giving them a higher cytotoxicity risk. Considering these outlines, NCs of poorly soluble compounds can be considered as safe, since most of the NCs based products are above 100 nm in size. Additionally, one of the important properties of NCs is that they are biodegradable and after addition of enough water, they just dissolve (and this is their purpose in the body). Each drug particle dissolving in the gastrointestinal tract will move from the meter to the nanometer size in the dissolution process. However, considering the aspects above, the NCs are belonging to the nanoparticles with best tolerability. The NCs are a priori low risk or non-risk nanoparticles; due to their particle size (generally

**Table 2. Available commercial products relying on drug NCs technology.**

S.No.	Brand Name (Chemical Name), Company Name	Production Process Involved	Route of Delivery	Uses	Problems with Old Treatment	Inference
1	<b>Gris-PEG*</b> (Griseofulvin) <i>Novartis Company</i>	Co- precipitation	Oral	Antifungal Agent	<ul style="list-style-type: none"> <li>➤ Oral griseofulvin is absorbed mainly from the duodenum.</li> <li>➤ Micro sized griseofulvin has a variable bioavailability (25-70%) and unpredictable oral absorption.</li> </ul>	Increased GIT absorption.
2	<b>Verelan PM*</b> (Verapamil)	Media milling	Oral	Anti- arrhythmic agent	<ul style="list-style-type: none"> <li>➤ To resolve the problems of verapamil solubility <i>via</i> oral route by maintain the low pH using organic acid.</li> <li>➤ To open pore in matrix at intestinal pH by addition of enteric materials.</li> </ul>	Increase oral bio-availability.
3	<b>Rapamune*</b> (Rapamycin) <i>Wyeth Pharmaceuticals</i>	Media milling	Oral	Immuno- suppressive agent	<ul style="list-style-type: none"> <li>➤ Poor solubility in water.</li> <li>➤ Do not produce optimum therapeutic effect.</li> </ul>	Require low dis- solution medium to solubilize NCs.
4	<b>Focalin*XR</b> (Dexmethyl-phenidate HCL) <i>Novartis</i>	Media milling	Oral	Psycho- stimulant agent	<ul style="list-style-type: none"> <li>➤ It has the elimination half-life in adults is variable with a mean of 3 hours.</li> <li>➤ It shows a bi-modal pharmacokinetic report that displays a peak at around 1.5 hours and a second peak at approximately 6.5 hours after administration.</li> <li>➤ So before complete absorption of drug from the site of administration the drug elimination gets started which cause to decrease the bioavailability profile of drug in body.</li> </ul>	Increase half-life of the drug for prolong release rate.
5	<b>Herbesser*</b> (Diltiazem) <i>Mitsubishi Tanabe Pharmaceuticals</i>	Media milling	Oral	Antianginal agent	<ul style="list-style-type: none"> <li>➤ It has problem of 45% bio-availability (first-pass hepatic metabolism) from the whole administered dosage.</li> </ul>	Increased bio- availability.
6	<b>Avinza*</b> (Morphine sulphate) <i>King Pharmaceuticals</i>		Oral	Psycho- stimulant agent	<ul style="list-style-type: none"> <li>➤ Bioavailability is approximately 30%.</li> <li>➤ Half-life is 2-4 hours.</li> </ul>	Increase half-life and bioavailability.
7	<b>Ritalin* LA</b> (Methyl- phenidate HCL) <i>Novartis</i>	Media milling	Oral	Psycho- stimulant agent	<ul style="list-style-type: none"> <li>➤ The absolute oral bioavailability of methylphenidate in children was <math>22 \pm 8\%</math>.</li> </ul>	Decrease peak plasma concentration time by increase in oral absorption.

(Table 2) contd...

S.No.	Brand Name (Chemical Name), Company Name	Production Process Involved	Route of Delivery	Uses	Problems with Old Treatment	Inference
8	<b>Zanaflex tm</b> (Tizanidine HCL) <i>Acorda</i>	Media milling	Oral	Muscle relaxant agent	<ul style="list-style-type: none"> <li>➤ It is slightly soluble in water.</li> <li>➤ Solubility in water decreases as the pH increases.</li> <li>➤ Absolute oral bioavailability of tizanidine is approximately 40%.</li> <li>➤ 95% of absorbed drug metabolizes.</li> </ul>	Increase solubility of drug in body.
9	<b>Emend*</b> (Aprepitant) <i>Merck &amp; Co.</i>	Media milling	Oral	Antiemetic agent	<ul style="list-style-type: none"> <li>➤ It is relatively lipophilic (log <i>P</i> at pH 7 is 4.8) and poorly water soluble at pH 2-10).</li> </ul>	Increase bioavailability due to small size.
10	<b>Tricor*</b> (Fenofibrate) <i>Abbott Laboratories</i>	Media milling	Oral	Hypercholesterolemia agent	<ul style="list-style-type: none"> <li>➤ Fenofibrate has low water solubility and high permeability.</li> <li>➤ The limiting step before the absorption is the drug dissolution.</li> </ul>	Increase dissolution profile of the drug.
11	<b>Triglide*</b> (Fenofibrate) <i>Skye Pharma</i>	High pressure homogenization	Oral	Hypercholesterolemia agent	<ul style="list-style-type: none"> <li>➤ It is insoluble in water.</li> <li>➤ Its bioavailability is optimized when taken with meals.</li> </ul>	Increase in bioavailability in fed condition also with increase in solubility in water.
12	<b>Megace ES*</b> (Megastrol acetate) <i>Par Pharmaceutical companies, inc</i>	Media milling	Oral	Appetite stimulant agent	<ul style="list-style-type: none"> <li>➤ The solubility of Megace® Oral Suspension is further reduced in the fasting state.</li> <li>➤ The bioavailability is decreased.</li> <li>➤ NCs formulation recommended taking the drug after a meal.</li> </ul>	Increase solubility and absorption rate in fed condition.
13	<b>Cesamet*</b> (Nabilone) <i>Elli Lilly</i>	Co- precipitation	Oral	Antiemetic agent	<ul style="list-style-type: none"> <li>➤ All the forms appeared to be equally hydrophobic and insoluble.</li> </ul>	Increased solubility of drug.
14	<b>Neprelan*</b> (Naproxen sodium) <i>Wyeth</i>	Media milling Media milling	Oral	Anti- inflammatory agent	<ul style="list-style-type: none"> <li>➤ Naproxen itself is well absorbed, but Food causes a slight decrease in the rate absorption.</li> <li>➤ The observed terminal elimination half-life is approximately 15 hours.</li> </ul>	Increase rate of absorption in fed condition.
15	<b>Theodur*</b> (Theo-phylline) <i>Mitsubishi Tanabe Pharmaceuticals</i>	Media milling	Oral	Bronchial dilator agent	<ul style="list-style-type: none"> <li>➤ Theophylline has extremely low aqueous solubility.</li> </ul>	Increase $T_{max}$ by increase in solubility profile of drug.
16	<b>Invega* Sustenna</b> (Paliperidone palmitate) <i>Johnson &amp; Johnson</i>	High pressure homogenization	Parenteral	Anti-depressant agent	<ul style="list-style-type: none"> <li>➤ Due to its low solubility, dissolution is slow.</li> <li>➤ Enables the delivery of the active drug into the systemic circulation.</li> </ul>	Increase in dissolution profile.

> 100 nm) and biodegradable nature (dissolution occurs in sufficient water amount). Consequently, the toxic risk of NCs is limited. However, NCs may cause undesired systemic effects in the body or intracellularly until the complete dissolution, which is generally not an issue because of the rapid dissolution behavior of NCs. The toxicity of the excipient material could be the main issue, not the toxicity of the NCs [97, 98, 109].

## **9. CHALLENGES TO OVERCOME THE PROBLEMS ASSOCIATED WITH NCS**

Despite the many benefits, drug NCs technology still has some drawbacks. Major one is the small size of NCs; they can enter any cell of body *via* pinocytosis and lead to further cytotoxicity. Second one is the new technique needs expensive equipment, increasing the cost for the final drug. Moreover, this technique still cannot be regarded as a universal approach because it is only applied on BCS class II only. Finally, the preparation and stability of different drug NCs vary based on molecular drug structure [99, 110-112].

## **10. REGULATIONS FOR NANO TECHNOLOGY-BASED PRODUCT**

### **10.1. In India**

Regulation in India is still at its initial stage. Efforts have been taken by Government of India to extend the research of nanotechnology in academic institutions, R&D and National laboratories through financial assistance. Nanotechnology Sectional Committee was formed in March 2007 by Bureau of Indian Standards (BIS) to consider the standardization for nano devices, sensors, transistors, initiators *etc.*

The organization did not include consumer products in their agenda as standards for these products require advanced technologies and trained personnel [100, 113].

### **10.2. In USA**

Nanotechnology was not regulated specifically by the USFDA in the past as it holds the view that it regulates products and claims regarding them but not technology. Further it believes that its existing technologies are self-sufficient for nano-

technology testing. It has placed nanotechnology in critical path project and wants to take the research done by a manufacturer to develop standards and testing procedure to verify their technology. USFDA has also constituted National Nanotechnology Initiative and Nanotechnology task force to evaluate the current regulatory approaches which will help in developing safe and effective products [114].

## **CONCLUSION**

Drug NCs are viewed as important amongst the most essential approaches for poorly dissolvable drug toward the start of this new century. The smartness of innovation is that it very well may be effectively applied to any drug. Indistinguishable to micronisation, it is a universal formulation principle, yet limited to BCS class II drugs. The primary preferred standpoint is that the NCs formulation can be applied to any route of administration that implies oral as well as parenteral, particularly I.V administration. Some other delivery route is dermal delivery to make supersaturated system with high thermodynamic movement, nasal administration route to stick NCs to the nasal mucosa, ophthalmic delivery to make system with prolonged retention times, vaginal delivery to make system equitably spread all through the therapeutic area, and pressurized aerosol products containing drug NCs for pulmonary administration. In spite of board applications of NCs, there are restrictions, similar to the requirement of the final product size on the hardness of the initial crystals and the contamination level they may bring into the NCs. Notwithstanding, a large number of these constraints were overwhelmed by the presentation of bottom up techniques. The most generally known bottom up method is fast precipitation. Nonetheless, control of particle size is a major issue in precipitation, and, for most particles or drugs, the crystal size can undoubtedly surpass a couple of micrometers. The precipitation was later improved by binding the organic molecule in a network to keep their unnecessary development or enlargement in size. Recently, another bottom up methodology dependent on seeding and mechanical pressure was developed to deliver natural NCs with a size of 100- 150 nm for a wide range of drugs. But all the reviews and studies show that solubility and

bioavailability problems of drugs could be solved by using NCs technology.

### LIST OF ABBREVIATIONS

BCS	=	Biopharmaceutics Classification System
BIS	=	Bureau of Indian Standards
FDA	=	Food and Drug Administration
HPH	=	High-Pressure Homogenization
MM	=	Media Milling
NC	=	Nanocrystal
RESS	=	Rapid Expansion in Supercritical Solutions
SCF	=	Supercritical Fluid
SEDDS	=	Self-Emulsifying Drug Delivery System
SFE	=	Spray Flash Evaporation
SMEDDS	=	Self-Micro Emulsifying Drug Delivery System
YMP	=	Yucatan Micropig Skin

### CONSENT FOR PUBLICATION

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