JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

A REVIEW ON NANOCRYSTALS: APPLICATIONS, PREPARATION, CHARACTERIZATION AND PATENTED DRUGS

Barmala Prashanth[1*], SK. Sushma Taj[2], Cheguri Shivani[3], Kotha Pranathi[4], Yellu Mahesh Reddy[5], Saduvu Shiva Kumar[6]
[1,3,4,5,6]Research students, [2]Assistant Professor,
Department of Pharmaceutics,
Joginpally B.R. Pharmacy College, JNTUH, Bhaskar Nagar, Yenkapally, Moinabad, Telangana, India.

Abstract:

Nanocrystals (NCs) are the class of solid dosage forms which utilizes the concept of nanoscience together with crystal nature of drug to achieve advantages in terms of solubility, dissolution, and physicochemical properties. Solubility has been a major problem for BCS classes II and IV. As a result, nanotechnology may be useful in resolving the solubility problem. This review will cover Nanocrystals, as well as the numerous methodologies employed in their formulation and their application. Nanocrystals are nanometre-sized drug particles of a poorly-water-soluble compound. They are distinct from polymeric nanoparticles in that they are entirely made up of the drug. Nanocrystals can be implemented to increase the rate of dissolution and saturation solubility of active pharmaceutical ingredients. Solubility has been a major problem for BCS classes II and IV. As a result, nanotechnology may be useful in resolving the solubility problem. This review will cover Nanocrystals, as well as the numerous methodologies employed in their formulation and their application. Nanocrystals are nanometre-sized drug particles of a poorly-water-soluble compound. They are distinct from polymeric nanoparticles in that they are entirely made up of the drug. Nanocrystals can be implemented to increase the rate of dissolution and saturation solubility of active pharmaceutical ingredients.

***Keywords*:** Nanocrystals, BCS class II, BCS class IV, micronization, nano-sizing, nanosuspension

I. INTRODUCTION:

The number of newly developed drug molecules display poor bioavailability because of low aqueous solubility. Nearly 40 percent new chemicals entities (NCEs) produced during drug discovery in last 25 years are poorly soluble in water [1]. For weakly water-soluble molecules, a more recent method of drug administration has drawn a lot of interest. The medication is delivered as particles or crystals of nanometer-sized which when surface stabilised can be used to prepare a colloidal solution. "Nano sizing" refers to the size reducing process of API to submicron level. Aqueous dispersions known as nanosuspensions are made up of a mixture of API and stabilisers, such as a polymer or surfactant in water. By providing electrostatic or steric repulsion on the surface of the nanoparticles, these stabilisers aid in preventing the clumping of the particles and maintaining their separation from one another. Nanotechnology is science and at nanoscale (10^{-9} m^2). The media milling method is usually used to create the nanoparticles. Nanometer-sized drug particles that can be disseminated in water and stabilised by surface ligands familiar with their surface are produced by the shear forces applied during the impaction of the milling process on the micron-sized drug crystals [2].

However, the "non-specific" methods can be used to increase the solubility of nearly any pharmacological molecule, with a few notable exceptions. Among the best applications of this vague formulation technique is the drug's micronization, which raises its surface area per unit volume and ultimately leads to a rapid rate of dissolution and high concentration of medicines at the place of absorption/action. Colloid mills and jet mills are widely utilized devices in several sectors for the purpose of medication micronization. Drug particles can be reduced by micronization to a size distribution range of 2 to 5 µm [3].

Pure solid drug particles with a mean diameter of less than 1000 nm are known as drug nanocrystals. Because drug nanocrystals are encapsulating carrier-free nanoparticles, they have the benefit of 100% drug loading. The medication and one or more stabilisers are distributed in aqueous or nonaqueous solutions in the formulation of nanocrystals. One or more widely accepted safe excipients (such as salts, sugars, buffers, or surfactants) could serve as stabilisers. It is possible to further post-process the liquid dispersion nanocrystals into solid or sterile injectable dose forms. Products containing nanocrystals have been found to have therapeutic uses in targeted drug delivery, oral, parenteral, ophthalmic, cutaneous, and pulmonary administration. There are multiple ways to give nanocrystals, unlike micronised medications. It can be administered orally as tablets, capsules, sachets, or powder; tablets are the preferred form. Because of their extremely small particle size, nano suspensions can also be given intravenously, increasing their bioavailability to 100%.

For oral drug particles that belong to the BCS class II and class IV drug categories, nanosuspensions have been recommended as a comprehensive delivery method [4]. The Developability classification system (DCS) was proposed by Butler and Dressman as a means of classifying substances in a manner that is more biorelevant way [5]. The intrinsic solubility and associated intra-luminal drug concentration for compounds classified as class II and IV are too low to even contemplate attaining adequate flux over the epithelial membrane, according to the DCS, which illustrates the differences between dissolution rate-limited and solubility-limited compounds. For molecules falling under DCS Class II b and IV, complexation or formulation techniques then rely on solid-state changes that may manifest differently in respect to nanocrystals [6,7].

Strictly speaking, if the drug is in amorphous form then the word "crystals" cannot be used to describe it but many publications in which drug molecules were confirmed to be in the partial crystalline state have used the term "nanocrystals" to describe it [8]. Preparations having drug molecules in other than pure crystalline forms are called as “amorphous nanoparticles” or “amorphous drug nanosuspension” [9].

METHODS OF MANUFACTURING

Different techniques have been explored for the manufacture of drug nanocrystals. The active pharmaceutical components are broken down into nanoparticles using mechanical forces as part of the milling operations, which also involve homogenisation.

There are several commercial items on the market that use this technique and have regulatory organisation’s approval. Nevertheless, these techniques generate nanoscale size by applying great pressure or energy. Further disadvantages of mechanical attrition include electrostatic effects, high energy consumption, time consumption, and little control over particle size.

The preparation of the nanocrystals via the crystallization process requires very little mechanical energy. The following procedures are part of the crystallization method: (1) dissolution; (2) crystal development; (3) nucleation; and (4) filtration and drying [10].

Additionally, a variety of crystallization procedures were employed to create the nanocrystals, including high gravity, cryogenic techniques, ultrasonication, supercritical fluid, and microemulsion methods. There are four main ways to make nanocrystals: (1) bottom-up; (2) top-down; (3) Combination method (4) Spray Drying. While the bottom-up method involves precipitation, the top-down method involves homogenization and milling [11,12].

Various methods of preparations are being employed for the preparation of nanocrystals based on techniques used to produce crystals in the nanometer size range which include mechanical disintegration and particle growth. There are three main methods used to prepare nanocrystals.

1. Top -down method
2. Bottom- up method
3. Combination method

1. Top-down method

The two main top-down techniques for creating drug nanocrystals are media milling and high-pressure homogenization. To shrink the particle size at a breakneck speed, a suspension is continually pushed to pass through a very small gap, typically around 25 µm, during the high-pressure homogenization process. Media milling is a technique used to attrite dispersed drug particles using milling media, such as pearls or balls made of ceramics, cerium, yttrium-stabilized zirconium dioxide, stainless steel, glass, or beads covered with highly cross-linked polystyrene resin [13,14]. The primary problem with the wet milling process that might contaminate the final product is the creation of residues from the milling material [15]. One of the biggest development issues for products utilizing nanocrystals is the detection and control of such contaminants during the production processes. Other critical factors that need to be considered during the milling process are the product temperature, the chiller temperature, and the time required to achieve a specific particle size [16].

a. Media filling

This process involves filling a chamber with the drug, stabilizer, dispersion medium (usually water) and milling media (beads or rods). Shear forces produced by the milling media's movement cause the drug particles to shrink in size. Better size reduction capacity results from an exponential rise in the number of contact sites available for grinding and dispersing as the milling media's effective surface area grows [18].

b. High Pressure Homogenization

The basic rule is high pressure, which is between 100 and 1500 bars. Without a doubt, we can transform micron-sized particles into nanoparticles under this pressure. Furthermore, it initially requires particles in the micron range of less than 25 µm. The objective is to obtain an example from the jet mill because it allows us to reduce the particle size to less than 25 µm. Additionally, the equipment can be used for both continuous and batch processes. Additionally, it has a 40 ml to 1,000 L capacity. Here, we must first transform the particles into a pre-suspension state. Because of particle collisions, there is strong pressure and significant shear, which reduces particle size. In this case, adding viscosity enhancers is necessary to increase the viscosity of the nanosuspension. Pressure and homogenization cycles are the two factors that require the most attention in this approach [19]. In order to increase the solubility and oral bioavailability of daidzein nanosuspension, Hui Wang et al., developed it utilizing a high-pressure homogenization process with stearic and electrostatic stabilizers[20].Ritonavir nanosuspension was made by Alptug Karakucuk et al., using a microfluidizer and high-pressure homogenization with HPMC and sodium dodecyl sulfate as stabilizers. This enhanced oral bioavailability in fed condition [21]. When compared to a coarse powder and physical drug mixes, the nanosuspension of ziprasidone created by Emine Tashan et al., using a microfluidizer had greater water solubility [22].Sumathi.R et al., used high-pressure homogenization to create a polymeric nanosuspension of naringenin, which resulted in a higher rate of dissolution and improved stability[23].

c. Microfluidizer technology

Micro fluidization works on the jet stream concept, which involves moving the suspension quickly through a specially designed "Z" or "Y" type homogenization chamber. Several flow direction changes in a "Z" type chamber cause particles to collide and produce shear force, which breaks them. The suspension stream is split into two streams in the "Y"-shaped chamber, which subsequently collide frontally [24].

d. Dissocubes Technology

To make the standard micro-suspension, the drug is first dissolved in an aqueous surfactant or polymer solution using an ultra-turrax stirrer or a Silverson homogenizer. It is then high-pressure homogenized using a piston-gap homogenizer to create the "pre-suspension." At 500 and 1500 bar of pressure, a procedure typically requires five to twenty cycles [25].

e. Nanopure technology

Nanopure technology, developed and owned by PharmaSol GmbH/Berlin, is another way to generate nanocrystals utilizing the piston-gap homogenizer. In non-aqueous or low vapor pressure dispersion medium, high-pressure homogenization effectively reduces particle size. When the final objective of the nanosuspension is to be transformed into conventional dosage forms, such as pills and capsules, this is particularly beneficial. For drugs that are sensitive to temperature, this method reduces the amount of water in the dispersion medium, which either eliminates the need for a drying step or permits it to be carried out under less severe conditions [26,27].

2. Bottom-up Method

a. Precipitation method

The precipitation technique was initially used in hydrosol technology, developed by List and Sucker. The owner of the patent was Novartis (previously Sandoz). In the nanoprecipitation process, crystalline or semi-crystalline drug particles are nucleated and then grow, while the dissolved medicine molecules expand. The drug is usually dissolved in a suitable organic solvent, such as acetone, tetrahydrofuran or N-methyl-2-pyrrolidone. Allowing this solution to reach a supersaturation level then gives the dissolved drug molecules the chance to nucleate. In order to create drug nanocrystals, an antisolvent must be added to this supersaturated solution while stabilizers such as polyvinyl chloride, hydroxypropyl methylcellulose (HPMC), and hydroxypropyl cellulose (HPC) are present. This will cause pyrrolidone (PVP), Tween 80 and Poloxamer 188 to nucleate and precipitate quickly[28,29]. The precipitation method can only be used for pharmaceuticals that are soluble in at least one solvent and that solvent is miscible with the selected antisolvent. Reducing the solvents used in the processes to a level that is acceptable in the finished product is an essential extra requirement. The bottom-up approach requires strict control over the manufacturing process and avoidance of recrystallization into the micrometer range [30].

3. Combination Method

a. Nanoedge Technology

The "Nanoedge Technology" hybrid method entails microprecipitation of the medication, followed by a high shear or thermal annealing step. In the first phase, either crystalline or amorphous medicine is used. Nanocrystals are created by the precipitation method combined with a suitable solvent-antisolvent combination. At this stage, the drug particles may be completely crystalline, partly crystalline, or completely amorphous. Annealing, which entails applying a single or recurring energy source (direct heat or mechanical stress) and then letting the particle relax, can transform thermally unstable particles into more stable forms. This energy reduction can be accomplished by transforming the solid shape from a less ordered structure to a more ordered lattice. An alternative method to achieve this stability is to rearrange the surfactant molecules at the solid-liquid interface. Energy can be added through sonication, homogenization, microfluidization, countercurrent flow homogenization, or other methods that produce impact, shear, or cavitational forces. Drug particles of this kind that precipitate have a propensity to grow and become bigger particles. Before the particles can get to this stage, the drug suspension is homogenized to preserve the range of particle sizes. Every precipitate particle is transformed into a crystalline state by the subsequent annealing process [31].

b. Nanopure XP Technology

Moschwitzer and Lemke created the Nanopure® XP technology in 2005. Its foundation is the modification of the starting material by an evaporation process followed by high-pressure homogenization. This technology's primary benefit is that, even for extremely hard crystalline material, it drastically lowers the number of homogenization cycles [32]. Since the solvents are entirely evaporated prior to the homogenization step, this process eliminates any worries regarding the toxicity of solvents and allows for the use of a broad variety of solvents.

Properties of nanocrystals

The ability of an oral formulation to dissolve a medication may be a decisive element. The same problem arises with many hydrophobic drugs, especially those that fall into the BCS class II category, which has very poor water solubility. At the maximum dosage strength, a medication is considered highly soluble in 250 ml or fewer of aqueous solutions with a p^H between 1 and 7.5[33]. This is a basic standard for determining solubility. A medication that is poorly soluble cannot dissolve completely in 250 ml of medium at the highest dosage strength in the p^H range of 1 to 7.5. The rate of absorption through the gastrointestinal tract is limited by the irregular and delayed disintegration of poorly soluble medications. Consequently, a lot of research is being done to make medications more soluble. When compared to powder pharmaceuticals with a micron size, the unique attribute of NCs shows several positive benefits on the performance of drugs that are less or poorly soluble. Increased bioavailability, faster absorption, reduced fluctuation in fed or fasted states, a rapid onset of action, and less variability between subjects all contribute to greater safety and efficacy [34,35]. This increase and enhanced saturation solubility, which ultimately lead to a higher drug concentration that reaches the blood vessel and gastrointestinal system, are caused by mechanisms of enhanced oral bioavailability, increased rate of dissolution, and improved bioattachment to the cell membranes/surfaces of NCs loaded with pharmaceuticals [36]. Despite their unique characteristics and simple structure, NCs can also be used in drug-targeting delivery systems [37]. The physiochemical properties of NCs are responsible for their exceptional performance.

Increased Dissolution Rate

One significant technique to enhance a medicine particle's surface area is to lower its size. The Noyes Whitney equation suggests that a higher surface area increases the rate of disintegration of the medication [38]. Thus, when a drug's dissolution is a rate-limiting issue, micronization is a common method to increase its bioavailability. Further improvements in the micronization process led to the development of the nanotechnology method, which raises the surface area and rate of dissolution of the nanoparticles.

Enhanced saturated solubility

The saturation solubility has a constant value and is influenced by temperature, dissolve media and chemical type. This is true for powdered drugs that range in size from micrometres to nanometres. However, the particle size and crystalline structure also affect the magnitude range of less than 1-2 μm. Saturation solubility and particle size are inversely correlated. Understanding the rate at which nanocrystals dissolve is possible thanks to the Noyes Whitney equation [39,40].

The dissolving velocity of nanocrystal medications is strongly proportional to their saturation solubility (C) and surface area (A). For instance, the dissolving velocity (dx/dt) increases as the surface area (A) and saturation solubility (C) rise.

$$DA/h * (Cs-Ct) = dx/dt.$$

Where, A is the surface area, h is the diffusional distance, Cs is the saturation solubility, Ct is the particle concentration, D is the diffusion coefficient, and dx/dt is the dissolving velocity. As the equation for Prandtl demonstrates, diffusional distance h is an essential component of the hydrodynamic boundary layer and has a major impact on particle size [41].

Stability

It was found that the nanocrystal suspension was stable since there was no Ostwald ripening process and the particles did not agglomerate [42]**.** A suitable stabilizer can be added, and different stabilizers, surfactants, and amphiphilic copolymers can be used to obtain the desired stability [43,44]**.** As homogenization progresses, the surfactants quickly disperse, adsorb on the surface of the crystal, and stabilize the system by creating a static and electrostatic barrier between the crystals [45]. The surfactant must have an appropriate affinity for the particle surface, among other conditions, in order to stabilize nanocrystals [46]. Furthermore, a surfactant should produce a homogenization process with a high enough diffusion rate. In order to maintain the necessary steric or electronic repulsion between the particles, the stabilizer content must be sufficient to cover the entire particle surface, but more stabilization does not always translate into a better outcome. Itraconazole, a drug-loaded nanocrystal that is poorly soluble in water, was created by Sun et al. using the homogenisation process. Three cationic polymers—polyethyleneimine, chitosan, and N-trimethyl chitosan—were examined for their effects on the properties of nanocrystals, and it was found that they support the synthesis of by functioning as steric and electrostatic stabiliser particles at the nanoscale. Consequently, the physical stability of nanocrystals prepared with cationic polymer was significantly enhanced [47,48]. A separate study by Deng et al., developed and evaluated paclitaxel nanocrystals to understand the structure and stability. Their Pluronic F127 desorption experiment CMC was used to understand the varied surfactant absorption attachment to the nanocrystal surface above and below. Their results indicate that pluronic binds to the NC surface very strongly below the CMC level, but it quickly binds to the NC surface above the CMC level and separates the surface when diluted. Higher temperatures tend to induce Pluronic F127 micellisation to occur more frequently because of the lower CMC [49]. Therefore, choosing the best surfactant is a crucial factor in determining the product's quality [50, 51].

Permeability

Because NCs may stick to the skin more easily, they aid in cutaneous delivery. Nanocrystals with a size of 200–300 nm help pass through epidermal membranes more easily. NCs between 200 and 300 nm in size can deposit these channels, which act as a depot formulation in which the drug can release into surrounding cells over a longer period of time. Through this size range, NCs showed better penetration through human dermal hair follicles [52]. Because of their diversity of particle sizes, poorly soluble medications can also be delivered transdermally. Lipophilicity, or the drug release rate, is the limiting factor even if poorly soluble drugs have a higher penetration rate. The follicular route allows particles smaller than 40 nm to penetrate the skin. However, the bigger particles exhibit poor skin penetration networks of Langerhans cells due to their strong epidermal connections. While particles between 500 and 750 nm exhibited high permeability in the hair follicle, particles bigger than 5 µm had very little penetration into the stratum corneum layer [53]**.** Castaneda developed a nanocrystal filled with atenolol using a factorial design technique. To investigate the intraduodenal permeability of the small intestine, goat is sacrificed, the permeability of pure atenolol and atenolol nanocrystals was assessed using a Franz diffusion cell. The permeability percentage of atenolol nanocrystals was shown to be much greater than that of pure atenolol based on the diffusion experiment [54].

Adhesiveness

Because of the variety of nanosizes, one unique property of nanocrystals is their adhesiveness. The increased adhesiveness through the mouth leads to better absorption [55]. Kinetics and adsorption isotherms are two possible approaches for investigating adhesion properties. The kinetics of adsorption are influenced by the particle size. Adsorbates that can flow through porous adsorbents have a unique adsorption isotherm in the small polystyrene latexes (230–670 nm). Additional adsorption can occur up to the saturation sites indicated by an isotherm plateau. Large-particle adsorbates, on the other hand, adsorb as Langmuirian type and form a surface monolayer that mimics a smooth surface [56,57].

APPLICATIONS

Oral Drug Delivery

Drug nanocrystals can significantly increase the bioavailability of poorly soluble medications taken orally. Both (1) enhanced solubility and dissolution rate and ii) bioadhesion to the gut wall account for the increase in bioavailability. The administration of drugs in nanocrystal formulations results in a large gradient of concentration between the GIT and blood arteries, which greatly enhances absorption and, consequently, bioavailability. One classic example is the neutral, weakly soluble medication danazol. Danazol conventional micro-suspension (200 mg, 10 µm) has an absolute bioavailability of just 5.2% in beagle dogs. An absolute bioavailability of 82.3% was attained when the drug was delivered as an aqueous nanosuspension (200 mg, 169 nm); at the same time, the Tmax decreased and the Cmax rose by 15 times [58]. Oral administration of amphotericin B in nanosuspension form

produced a substantial improvement in its oral absorption compared to orally administered conventional commercial formulations such as Fungizone, AmBi-some and micrometer amphotericin B [59].

In order to improve its dissolution behaviour in different dispersion media, rutin nanocrystal-loaded tablets were created using a direct compression method. The dissolution velocity of the rutin nanocrystal-loaded tablet was found to be superior to that of tablets containing rutin microcrystal and the marketed formulation. After 30 minutes, the tablets containing rutin nanocrystal released nearly 100% of the rutin in water, whereas the microcrystal tablets and the marketed tablet only released 71% and 55% of the total amount of rutin, respectively [60].

To enhance its oral absorption, lutein nanocrystals were created. For usage in nutraceuticals, the nanosuspensions were transformed into dry products by combining them into pellets that were put inside hard gelatin capsules. Compared to coarse powder, the produced formulation showed a better in vitro release that was three to four times greater. After being lyophilized, the same lutin nanosuspension was added to gels and creams. When lutein nanocrystals were tested for permeation across cellulose nitrate membranes, their penetration was 14 times greater than that of the coarse powder [61]. Zhang, Lv et al., used a combination of high-pressure homogenisation and anti-solvent recrystallisation to create the bai-calein nanocrystal. The produced nanocrystals exhibited a significantly faster rate of dissolution and a mean relative bioavailability that was 1.67 times greater than that of baicalein crystals [62].

The probe sonication technique was used to create the fenofibrate nanocrystals. In 1% of sodium lauryl sulphate medium, the final formulation and pure medication are dissolved in-vitro with 73.89 percent and 8.53 percent, respectively. In comparison to the pure drug formulation, the final formulation's relative bioavailability was approximately 4.73 times greater [63]. In order to increase the mucoadhesiveness, buparvaquone nanosuspension was prepared and entrapped in the hydrogels. It was found that the incorporation of the nanosuspension into mucoadhesive hydrogels significantly improved the system's physical stability when compared to un-trapped nanosuspensions [64].

During the in-vitro dissolution test, the nanosuspension demonstrated a significantly higher dissolution rate than coarse suspension, and in the in vivo evaluation, the nanosuspension demonstrated a significant increase in $AUC_{0\text{-}t}$ and C_{max} relative to coarse suspension, while a decrease in T_{max} [65].

Parenteral drug delivery

Drugs administered by a variety of parenteral routes, including intravenous, subcutaneous, intramuscular, intra-articular, and intraperitoneal, can be made more efficacious by nanocrystals. A variety of excipients, such as co-solvents and surfactants, are required for an intravenous formulation of poorly soluble medicines in conventional drug delivery systems. However, they generate a number of negative responses and raise the dosage volume [66]. Nanocrystals can be administered intravenously with a lower dosage, a quicker start of effect, and the highest possible bioavailability because of their tiny particle size [67,68]. The NC size for the parenteral administration route should be less than 100 nm [69]. The intraperitoneal route can be used to successfully administer a variety of appropriate nanocrystals. In terms of lowering the median tumour burden, paclitaxel nanosuspension shown encouraging results when compared to pure taxo l [70]**.** When compared to liposome clofazimine, nanosuspension clofazimine, a poor water-soluble antileprotic medication, also exhibits improved stability and effectiveness in female mice infected with Mycobacterium avium [71].

Pulmonary drug delivery

Drug nanocrystals also offer good tissue adhesiveness and longer residence duration at the absorption site when administered via the pulmonary route, in addition to the benefit of a faster rate of breakdown. Curcumin-spray-dried powders for inhalation (curcumin-DPI) were created by wet milling curcumin nanocrystals. Curcumin-DPI exhibited a much higher rate of dissolving than coarse powder, according to the dissolution study's findings. The in vivo tissue distribution investigation revealed that the lung accumulated the majority of the curcumin-DPIs, which decreased the amounts in other tissues. Since the majority of the curcumin-DPIs were solely deposited in the lung, the plasma curcumin concentration attained by inhalation was, finally, noticeably greater than that obtained by oral route [72].

Hu et al. produced various sized Curcumin acetate nanocrystals and microparticles using the wet milling process. These were then spray-dried to produce an inhalable powder. Aerosolised curcumin acetate nanocrystals with a mean size of 123.7 nm had longer lung retention duration and AUC value 7.62 times greater than that of the microparticles, according to the biodistribution data. Additionally, curcumin acetate nanocrystals enhanced the availability of converted curcumin by 25.1 times and the local in vivo release rate by 3.3 times [73].

Ostrander et al., used NanoCrystal® technology to create beclomethasone dipropionate nanocrystals with 2%w/w Tyloxapol. When beclomethasone dipropionate was aerosolised using an Omron NE-U03 ultrasonic nebuliser, the 1.25% w/w colloidal dispersion produced a respirable drug dose of 22.6 to 39.4 mg per 2-second activation period, as opposed to 12.8 mg for a single activation of a Vanceril-marketed product. The respirable fraction varied between 56 and 72 percent for the nanocrystalline formulation and 36 percent for the propellant system when expressed as a proportion of the dosage that was released (via the mouthpiece or actuator) [74].

The anti-solvent recrystallisation and high-pressure homogenisation methods were used to create the baicalein nanocrystals. Pharmacokinetic characteristics of baicalein crystals and nanocrystals following gavage and pulmonary delivery were compared in an in vivo test conducted in rats. The pulmonary baicalein nanocrystals exhibited nearly identical pharmacokinetic properties to intravenous baicalein administration, as well as fast and widespread absorption [75].

Budesonide is an anti-inflammatory corticosteroid that was created as a high-pressure homogenisation nanosuspension. The combination of tyloxapol (a steric stabiliser) and lecithin (an electrostatic stabiliser) stabilised the budesonide nanosuspension. To nebulise the produced nanosuspension, a commercial nebuliser was used. According to photon correlation spectrophotometry, the nebulised nanocrystals' particle size was around 496 nm. For a year, the budesonide nanosuspension remained stable at room temperature and demonstrated safety and efficacy in healthy participants [76].

Ophthalmic drug delivery

The physiological barriers in the eye and the significant pharmacokinetic environment make ocular delivery a challenging system [77]. The most common and noninvasive medication delivery technique for addressing anterior segment eye issues is topical application [78]. One of nanocrystals' benefits is their long residence periods, which are essential for effectively treating most eye disorders. It is also not very tonic and the degree to which the drug dissolves in the lachrymal fluids determines how effective it is [79]. Consequently, the drug's intrinsic rate of solubility in lachrymal fluids determines both its bioavailability and ocular release. Particles contained in glucocorticoid nanosuspension, such as hydrocortisone, prednisolone, and dexamethasone, showed enhanced absorption and extended pharmacological activity [80].

Targeted drug delivery

The primary accumulation of the medication inside a zone that is particularly targeted for treatment is made possible by the targeted drug delivery. Specific engagement with a receptor in specified tissues is demonstrated by nanocrystals employed for targeted drug delivery. Four fundamental requirements must be met for targeted medication delivery to be effective: contain, evade, target, and release [81]. IV-injected nanocrystals disperse more in organs with MPS cells, such as the liver, spleen, and lung, than their solution counterpart because they are sequestered and transported by MPS cells [82, 83]. On the surfaces of nanocrystals, additional functional groups and targeted ligands can be introduced. In this manner, nanocrystals may be incorporated into different matrix architectures and directed to behave in a particular tissue or organ. RBC-NCs, or RBC membrane-coated drug NCs, were developed by Chai et al. and demonstrated a high drug load, superior biocompatibility, sustained stability, and extended retention period. In general, RBC-NC therapies can be utilised to treat different kinds of cancer and deliver different medications [84]. Using a LecA-deficient Pseudomonas aeruginosa, Hou created another targeted delivery method in which glycosylated copper sulphide nanocrystals demonstrated exceptional selectivity towards LecA and destroyed the bacteria in a synergistic manner with photothermal therapy. In addition to providing imaging, this innovative therapeutic system may be used to a variety of illness therapies especially cancer.

Toxicity

Despite size, there are a number of additional factors that might have a harmful impact on the body. Important characteristics that influence toxicity include surface charge, surface area, reactivity, chemical composition, shape, potential and solubility [85, 86]. The pulmonary system, oral and topical routes and the ocular route are some of the traditional ways that nanocrystals might be delivered. Compared to micronised medication particles, the particles could interact with the bodily tissues in a different way. Compared to big particles, nanocrystals dig deeply into bodily tissues. The nanocrystals' capacity to evade the reticuloendothelial system also allows them to stay in the body for a longer period of time. Particles of a lower size will have a longer retention period in the body. In addition to their phagocytic activity, only a limited percentage of cells are able to absorb NCs with sizes between 100 and 1000 nm. Particles less than 100 nm are absorbed by all cell types via the endocytosis mechanism, hence are considered riskier [87, 88]. The size-related risks and bio-persistency are used for classifying nanoparticles. Classification according to the risk of toxicity: class I (>100 nm, biodegradable), class II (>100 nm, non-biodegradable), class III (< 100 nm, biodegradable), and class IV (< 100 nm, non-biodegradable). Class-I NCs are regarded as harmless because of their biodegradable nature and particle size more than 100 nm, which also results in little or nonexistent negative effects. Nonetheless, the harmful impacts of NCs are often minimal. Unwanted impacts on the body's blood circulation may result. As a result, NC development requires thorough research to facilitate possible outcomes while minimising hazardous consequences, especially when using biodegradable nanoparticles. Generally speaking, nanocrystal medications are safer and more tolerated across a range of delivery methods than traditional pharmaceuticals [89].

CHARACTERIZATION OF NANOCRYSTALS

Characterization of the formulation is as vital for the effective production of a nanocrystal formulation as the selection of the right excipients. This is done to make sure that the parameters that are responsible for the performance of the nanocrystals are within the designated limits. A detailed discussion of the many characterisation tests used to assess nanocrystals is provided in the sections that follow.

Solid state properties

The degree of crystallinity, solvate (particularly hydrate) form, and polymorphic crystal shape are solid state characteristics that affect perceived solubility and, consequently, the rate of dissolution. Therefore, it is essential to identify these properties in nanocrystals. Preventing solid state transitions during manufacture, storage, and/or administration is the goal of the thermodynamically most stable crystalline form. The resulting solid state form can be affected by various nanocrystal production circumstances and processes. Furthermore, the polymorphic form that is thermodynamically stable is influenced by the surrounding environment. Hydrate forms, for instance, are often less soluble in aqueous conditions due to their increased stability [90].

Thermal analysis

Drug and drug nanocrystal thermal behaviour is frequently studied using differential scanning calorimetry (DSC). Following nanocrystal synthesis, DSC experiments are conducted to assess the drug's crystallinity and the interaction between the drug and excipients. This has particular significance for medications that exist in several polymorphic forms. Increased saturation solubility can also result from some top-down methods, such as high pressure homogenisation, which can produce particles with an amorphous proportion. Pure drug, drug and excipient (stabiliser) physical combination and final formulation (which may be in dried form) are all subjected to DSC. Heat flux DSC and power compensated DSC are the two forms of DSC that may be distinguished by their modes of operation. Two pans—a sample pan and an empty reference pan—are set on a thermoelectric disc that is encircled by a furnace in heat flux DSC. The thermoelectric disc transfers the heat from the furnace to the sample and reference pan at a linear heating rate [91, 92, 93]**.** However, the sample's heat capacity (C_p) would cause a temperature difference between the sample and reference pans, which is detected by area thermocouples. The thermal equivalent of Ohm's equation, $q = \Delta T/R$, would then calculate the resulting heat flow. Where, sample heat flow is denoted by q, temperature difference between sample and reference by T, and thermoelectric disc resistance by R [94]**.** The sample and reference pans are kept in different furnaces that are heated by different heaters in a power-compensated DSC [95, 96]. After keeping the reference and sample pans at the same temperature, the difference in thermal power needed to keep them there is calculated and shown against either time or temperature. Kocbek et al. used Poloxamer 188 as a stabiliser to create an ibuprofen nanosuspension. The DSC curve of pure ibuprofen showed a single exothermic peak at an onset temperature of 74.8 °C because to its melting. With a starting temperature of 51.4 °C, Poloxamer 188's DSC curve also shows a single endothermic peak. The DSC curve of the freeze-dried ibuprofen-Poloxamer 188 nanosuspension showed two different endothermic shifts. The first endothermic change appears as a tall narrow peak with an onset temperature at 39.4 °C and the second as low broad peak with temperature of maximum at 56.8 °C, where it was not possible to analyze the onset temperature. These results indicate formation of eutectic mixture of drug and Poloxamer 188. In this study, the peak at the lower temperature represents eutectic system melting and change at the higher temperature, represents excess component melting. Based on second peak position, it can be estimated that surplus component left is ibuprofen. Therefore, the increased drug dissolution rate is explained by the formation of teutectic system and submicron-sized drug crystals produced during nanosuspensions formulation [97].

Among other thermal methods, hot stage microscopy, sometimes referred to as thermal microscopy or thermomicroscopy, combines thermal analysis with microscopy to allow for the physical characterisation and study of materials as a function of time and temperature. In addition to helping with polymorph screening and characterisation, hot stage microscopy also helps distinguish between the crystalline and amorphous regions of nanocrystals. By spray-drying a novel drug moiety, BMS-347070, with a surfactant, Pluronic F127, Yin et al. created nanocrystals. The drug processed with Pluronic F127 and the micronised pure drug were compared by the authors using hot-stage microscopy. The co-processed and micronised drug's hot stage microscopy pictures were compiled at a magnification of 100X. The two samples were placed in the same area of view on the slide. At a rate of 10°C per minute, the slide was heated to 250°C. Compared to the pure drug, whose size cannot be ascertained by optical microscopy, the drug particles that remained in the molten Pluronic were discovered to be much smaller [98].

Differential thermal analysis (DTA) or thermogravimetric measurements can also be used for thermal analysis. Huang et al. applied DTA to the thermal analysis of SKLB610 nanosuspensions at a heating rate of 10°C/min in the range of 25–600°C. The DTA curve of SKLB610 showed a drug melting peak at 155.7°C, but no such peak was seen with the nanoparticles. Pure SKLB610 gave peak at 132–133°C. The melting peak of SKLB610 in nanosuspension was relatively blunt in comparison to that of pure SKLB610, presumably because of the preparation process [99].

X-ray diffraction

Following a drug's conversion to a nanocrystal formulation, X-ray diffraction studies are typically conducted to confirm the drug's crystallinity. A diffraction pattern is produced when X-rays interact with a crystalline substance; each crystalline substance in a mixture of substances produces its own pattern independently of the others, and the X-ray diffraction pattern of a substance thus represents the substance's unique fingerprint. The authors used XRD to analyse the change in the crystalline nature of the drug after it was converted into nanocrystals.

Furthermore, compared to the pure drug, the powder XRD analysis of the spray-dried nanosuspension made using a top-down method (high speed milling) revealed a slight shift in the major peaks. At the same 2θ values, the distinctive peaks for the milled and unmilled drug were seen. When spray-dried nanosuspension was run at a higher milling speed, a small reduction in peak intensity was noted [100].

FT-IR studies

FT-IR investigations are used to assess the chemical characteristics of the medicine and its interactions with excipients. Curcumin nanocrystals were created and assessed by Liandong et al. for pulmonary administration. To assess the change in the medication's chemical characteristics, FTIR analyses were performed on both the pure drug and the dry powder inhalation that was created (wet milling followed by spray drying) [101].

Raman spectroscopy

Raman spectroscopy is a spectroscopic technique that relies on the inelastic scattering of monochromatic light from a laser source, which means that after interaction with a sample, the frequency of the photons in the monochromatic light changes. The frequency of the reemitted photons is shifted up or down from the original monochromatic frequency. In this process, the size of nanocrystals was influenced by factors like the freezing rate. Therefore, the crystallisation process was monitored by Raman spectroscopy to ascertain the stage at which the solutes crystallised and how the freezing rate affected the particle size [102].

Techniques based on liquid atomisation, such as spray drying or electrospraying, are particularly prone to producing a final product that is partly or completely amorphous. However, annealing can be used to achieve complete crystallinity following manufacture. Polymorphic alterations may also be brought on by the high shear stresses connected to high-pressure homogenisation and wet medium milling [103].

Wet ball milling was utilised to create the nanocrystals, and poloxamer 188 was added as a stabiliser. When the identical milling procedure was utilised, there were no appreciable changes in the particle sizes of the two polymorphs; nevertheless, over the ninety days of stability testing, there were variations in the stability concerning the particle size. The drug's polymorphic form was unaffected by the grinding. The milled polymorphs' crystallite size was determined using XRPD peak width broadening. The size of the crystallites was found to be around 90 nm for polymorph 1 and 65 nm for polymorph 2 [104].

Particle size and size distribution

Because they influence other characteristics including physical stability, saturation solubility, dissolving velocity, and even therapeutic effectiveness, size and size distribution are crucial indicators of the nanosuspensions. Particles with lower sizes have greater surface energies, which encourages aggregation. Microscopy, dynamic light scattering, and static light scattering are the most widely used methods for measuring the particle size of nanoscale systems. Dynamic light scattering, sometimes referred to as photon correlation spectroscopy (PCS), is commonly used to determine the mean particle size of nanosuspensions[105]**.** Its benefits include measuring quickly and easily and producing precise findings. Nevertheless, particles bigger than 6µm cannot be analysed using this method. The breadth of the particle size distribution, also known as the "polydispersity index" (PI), may be obtained from PCS in addition to the mean particle diameter. One important metric that controls the physical stability is the PI value, which goes from 0 (monodisperse particles) to 0.500 (wide dispersion). For stability over the long run, the PI should be as low as feasible.

Particularly for nanosuspensions intended for parenteral and pulmonary distribution, optical microscopy and low angle static light scattering (also known as laser light diffraction) are methods for detecting bigger particles. Although light microscopy has the benefit of being visible and producing results that are indisputable, a significant disadvantage is that it lacks statistical significance since it is impossible or extremely time-consuming to analyse 10,000 particles or more, which is required for a proper study. The ability to analyse big particles, tiny nanoparticles, and mixes of small and large particles in a single measurement makes laser diffractometry (LD) a reliable method that outperforms all others. Depending on the kind of apparatus used, the LD produces a volume distribution and has a measurement range of around 0.05–80 µm up to a maximum of 2000 µm. D50, D90 and D99, which indicate that 50% of the particle volume is below the specified size, are typical characterization characteristics of LD. These diameters are 50%, 90% and 99%. When it became necessary to analyse nanoparticles, the drawbacks of laser diffraction techniques

increased because the technology was initially designed to analyse bigger particles in the micron range. It was intended to expand the measurement range (for example, from 400 nm to 2000 nm) to a wider range, as laser diffraction is a quick and easy technique that can analyse even extremely small particles (for example, from 20 nm to 2000 nm). However, in practice, this approach can only be used to analyse particles that are 400 nm or bigger [106].

Since the intensity of diffracted light declines with decreasing size, laser diffraction is not practical for measuring particles smaller than 400 nm. Modern LD devices, however, are capable of analysing particles as small as 20 nm and as large as microns. By adding a second, supplemental approach to the experiment, the measuring range for extremely minute particles might be expanded. By identifying other optical events, such as scattering intensities in various directions, the supplementary approach learns more about the particles [107, 108]. As a result, the extra methods differ from diffraction of pure laser light. A pooled result of pure LD and supplementary technique is obtained by combining the extra data from the supplementary technique with the size analysis of the LD measurement. Thus, in a strict sense, the LD measurements of today are a combined report of two distinct methodologies rather than just pure LD observations. However, by ignoring bigger particles (such as massive crystals and/or aggregates/agglomerates), the additional approach may likewise overstate the presence of the nanoparticles [109]. Since LD is often employed to identify potential huge particles in addition to a main bulk population that is nanosized, or to demonstrate the lack of such large crystals, which is not achievable with PCS measurements, this observation is quite significant. In conclusion, only when all of the previously described factors are taken into account can particle size of nanocrystal formulations provide significant outcomes. Furthermore, it should be mentioned that the particle size data of a nanosuspension acquired by PCS and LD differ from one another since the PCS mean particle size is a light-intensity weighted size, whilst the LD data are volume based. In order to administer nanosuspensions intravenously, a Coulter counter analysis is required. The Coulter counter results provide an absolute value, or the absolute number of particles per volume unit for the various size classes, in contrast to the volume distribution of the LD analysis. Since the smallest blood capillary is just 5 µm in size, even a trace amount of particles larger than that might result in emboli or capillary blockage. Therefore, Coulter counter analysis should be used to rigorously regulate the amount of microparticles in nanosuspensions [110].

The substance to be analysed presents challenges in particle size analysis in addition to the experimental setup. According to general theory, the most crucial need for accurate and repeatable results is the sample's stability throughout analysis [111]. This is not always simple to do, though, and occasionally the changes are not even discernible. Agglomeration and dissolution are two examples of potential sample changes or instabilities. As a result, materials with high solubility and/or enhanced dissolution velocity may be particularly vulnerable to these changes during analysis.

In addition to these, scanning tunneling microscopy, scanning probe microscopy, and confocal laser scanning microscopy are employed for the particle size investigation.

Particle shape and morphology

Ideally, a scanning electron microscope (SEM) or a transmission electron microscope (TEM) may be used to identify the morphology or form of the nanocrystals. The TEM analysis requires a moist sample with an appropriate concentration. A SEM examination is essential to track changes in the size and shape of the particles both before and after the water removal procedure when the prepared nanosuspensions are to be dried into a powder (for example, by lyophilization or spray drying) [112].

A type of scanning probe microscope called atomic force microscopy (AFM) uses a probe to evaluate local characteristics including height, friction, and magnetism [113].

Studies of interactions between solid drug surfaces and aqueous stabiliser solutions have used surface plasmon resonance (SPR) analysis. Indomethacin nanocrystals were stabilised using five structurally distinct PPO/PEO block co-polymers, and contact angle and SPR measurements were utilised to ascertain the affinities of the stabilisers with solid drug surfaces [114].

When the nanocrystals are to be made into dry powder inhalers (DPIs) for direct medication administration to the lungs, particle shape is crucial.Van der Waals forces, which include particle surface morphology, size, shape, electrostatic characteristics, and hygroscopicity, are associated with particle interactions. Because they are less likely to combine, particles with limited contact area and van der Waals force can spread out easily in the atmosphere. Because of their strong attraction forces, elongated particles are not the best for aerosolization [115].

Particle surface charge

One of the elements affecting the physical stability of nanosuspensions is the particle surface charge. The electrical repulsion between the particles and their physical stability increase with the degree of particle equality. The "zeta potential," which is determined by measuring the particles' electrophoretic mobility in an electric field, is the optimal way to quantify the particle surface charge. Colloid titration can be used to assess the particle charge in surface charge per unit [116, 117].

Ions from the dispersion medium adsorb onto the particle surface in an electrolyte-containing solution. A negative Nernst potential is assumed for the explanation of this model. Generally speaking, negatively charged, fixed, and dehydrated ions make up the first absorbed monolayer of ions, also known as the Helmholtz layer. The measurement is a particle electrophoresis, and the Doppler shift of the laser light scattered by the moving particles is used to calculate the particle velocity.

Typically, a field strength of 20 V/cm is employed. The Helmholtz–Smoluchowski equation was used to translate the electrophoretic mobility into the zeta potential in millivolts. This equation may be reduced to multiplying the observed electrophoretic mobility (μm/cm per V/cm) by a factor of 12.8 to obtain the ZP in mV under typical measurement circumstances (room temperature of 25ºC, water) [118, 119].

Dissolution of nanocrystals

The solubility of the drug's most stable crystalline form in a particular medium at a certain temperature and pressure is implied by thermodynamic solubility. For a brief period, solubility may surpass thermodynamic solubility. This can be seen in medication particles that are nanosized, amorphous, or metastable polymorphism. Various concepts, including kinetic and perceived solubility, have been used to describe this improved solubility [120]**.** While the equivalent results for drug nanocrystals were 67.51 and 107 μg/mL, respectively, the bulk drug's thermodynamic solubility in aqueous 0.5% and 1% sodium dodecyl sulphate solution was 6.02 and 23.54 μg/mL [121].

Particle size and stabiliser had a significant impact on intrinsic dissolution rates. The intrinsic dissolution rate for the smallest nanocrystals (580 nm) using poloxamer F68 as a stabiliser was 0.50 μg/min/mm^2, whereas poloxamer F127 had a rate of 0.31 μg/min/mm^2. Additionally, the rate at which bulk indomethacin dissolved was measured and found to be much lower at 0.05 μg/min/mm^2. UV imaging has also been used to determine concentrations on the surface.

It was discovered that the model medication, indomethacin, interacted with the material of the centrifuge tube and the kind of filter that was examined. Several wavelengths can be used for the analysis in order to identify any undissolved drug particles in the sample [122].

A variety of methods may contribute to the polymer's parachute effect. Initially, the polymers themselves can improve the drug's thermodynamic solubility (also known as the co-solvency effect), which reduces supersaturation and, in turn, the thermodynamic driving force for crystallisation (this also creates an extra spring effect with the polymer). Even the addition of trace quantities of polymers like PVP and HPMC to solution can greatly improve the water solubility of the medicine through drug-polymer interaction in solution via electrostatic interactions, van der Waals forces, or hydrogen bonding [123].

Permeation Study

Drugs with limited solubility may benefit greatly from improved cutaneous bioavailability using nanocrystal-based drug delivery. Indeed, the nanocrystal has the ability to be more sticky to the skin, which facilitates dermal distribution, in addition to having higher saturation solubility and dissolution rate. Drug delivery to the skin occurs via two mechanisms: the first is a straightforward rise in the concentration gradient between the formulation and the skin, and the second is mediated by hair follicles. These shunts serve as a depot from which the medication may diffuse into the surrounding cells for prolonged release, and nanocrystals of the right size (about 700 nm) can deposit into them [124].

Medication retention and penetration into the eye can be enhanced by using nanocrystal-based medication delivery. Increasing solubility in lachrymal fluid and producing sticky qualities are two potential mechanisms for this. The type of the surfactant in the formulation determines the adhesive characteristics that nanocrystals can provide, which can be employed to improve medication retention and penetration into the eye in addition to increasing solubility in lachrymal fluids of poorly soluble medicines. Because they are often less irritating, non-ionic surfactants are chosen over ionic ones. Typically, the Franz diffusion cell equipment is used for the permeation research [125, 126]**.**

Drug absorption from nanocrystalline formulation

Both solubility and permeability have a direct relationship with drug absorption, but lipophilicity has an inverse relationship. Following dissolution from nanocrystals, the dissolved medication passes through the intestinal wall (much as a medicine from a solution formulation). Stabilisers in the formulation itself interact with cells of the epithelial layers to facilitate permeation, in addition to boosting drug penetration through increased dissolved concentrations.

They investigated five distinct medications and created nanocrystals by homogenising them under high pressure using the same stabiliser, poloxamer 188. All of the medicines that were examined had particle sizes of around 430 ± 30 nm. In every instance, the AUC values were between 1.4 and 7.2 times greater than those of drug microsuspensions given to rats orally. It was discovered that

polar surface area, log p value, and melting point all affected medication absorption. With nanocrystals of the same size, drugs with low melting points, log p values of around 5, and polar surface area values of 50 to 60 showed better absorption [127].

Gao and colleagues assessed the ability of TPGS stabilised paclitaxel nanocrystals to overcome P-glycoprotein drug-resistance in P-gp overexpressing H460 cancer cells. It was discovered that the stabiliser TPGS effectively reduced the drug resistance of the cells under study. It is well known that after an intravenous injection, drug nanoparticles build up in the tumour tissues as a result of the increased permeability and retention (EPR) effect.

Label-free imaging of nanocrystal-cell interactions may be possible using the new label-free, chemically selective, and non-destructive techniques of confocal Raman microscopy and coherent anti-Stokes Raman scattering (CARS) microscopy. If a submicron particle has a strong enough Raman or CARS signal, it may be analysed using these methods (the resolution and speed are greater for the intrinsically confocal CARS approach, while the chemical specificity is better for Raman microscopy).

The CH_2 stretching resonance, which is primarily linked to the palmitate moiety, was used to image the nanocrystals in both fixed and living cells. In this instance, the nanocrystals were separated from the endogenous lipid by geometrical differences, and an otherwise weak lipid signal from the cells was used; however, for chemical specificity, a CARS resonance separated from lipid signals could be used with other drugs. Intracellular nanocrystals were seen in the granulomatous tissue in tissue sections [128, 129].

CONCLUSION

For the formulation of medications that are poorly soluble, nanocrystal technology appears to be a potential technique. Even though they are poorly soluble, the nanocrystals simply dissolve and vanish when a significant volume of water is present. They have been effectively used to increase medication bioavailability, target drugs more precisely with fewer adverse effects, lower dosages, and ultimately increase patient compliance. They can be included in more patient-friendly solid dose forms like pills and capsules. However, further verified therapeutic evidence is still pending and their nanotoxicity has to be evaluated. Nevertheless, nanocrystals have the potential to be used in a wide range of upcoming medicinal and cosmetic goods. When it comes to cancer therapy, they might be viewed as a glimmer of hope.

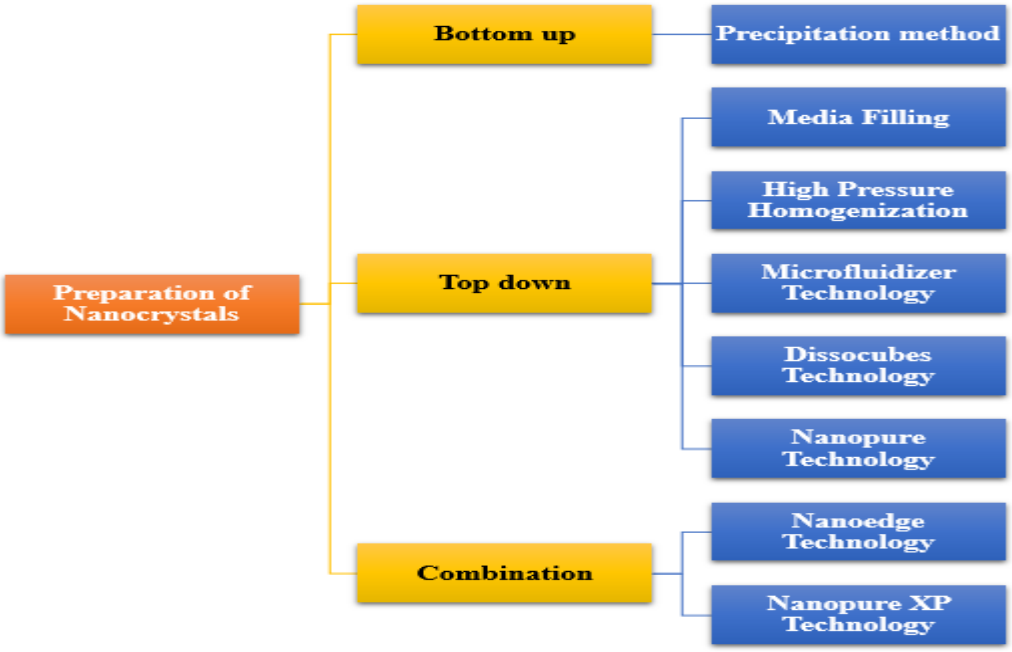

Fig. 1 Different methods of preparation of Nanocrystals.

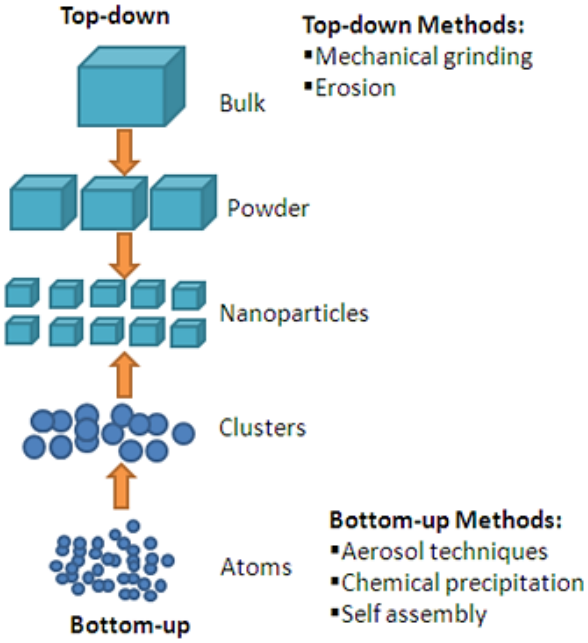

Fig. 2 Top-down and Bottom-up methods of preparation of Nanocrystals.

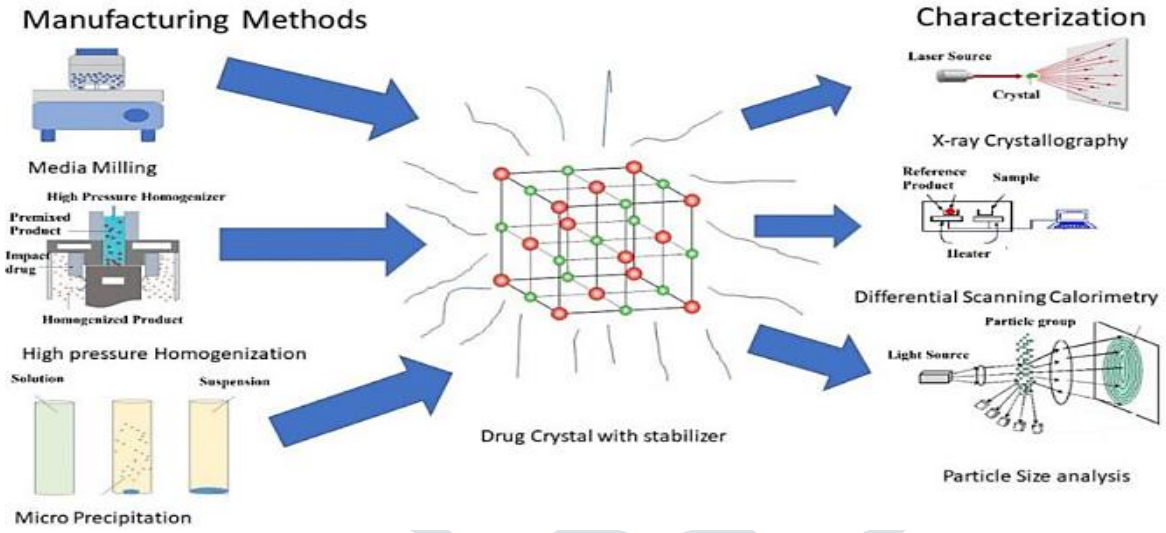

Fig. 3 Manufacturing methods and characterization of Nanocrystals.[17]

Nanocrystals drug products in clinical trials:

Table 1 Nanocrystals drug products in clinical trials [130]

Trade name	Company	Drug	Indication	Technology	Delivery route	Approval year
Rapamune	Wyeth	Rapamycin/ sirolimus	Immunosuppressive	Coprecipitation	Oral	2000
Ritalin LA®	Novartis	Methylphenidate hydrochloride	Anti-psychotic	Media milling	Oral	2002
Avinza®	King Pharma	Morphine sulfate	Anti-chronic pain	Media milling	Oral	2002
Emend	Merck	Aprepitant	Anti-emetic	Media milling	Oral	2003
Tricor	Abbott	Fenofibrate	Hypercholesterolemia	Media milling	Oral	2004
Megace®ES	Par Pharma	Megestrol acetate	Appetite stimulant	Media milling	Oral	2005
Triglide	Skye Pharma	Fenofibrate	Hypercholesterolemia	High pressure homogenization	Oral	2005
Naprelan®	Wyeth	Naproxen sodium	Anti-inflammation	Media milling	Oral	2006
Invega Sustenna®	Johnson & Johnson	Paliperidone palmitate	Antidepressant	High pressure homogenization	Parenteral	2009
Cesamet®	Lilly	Nabilone	Anti-emetic	Coprecipitation	Oral	2009

References:

1. Florence, Alexander. 2008. Nanoparticulate Drug Delivery Systems, D.ThassuM.DeleersY. Pathak Informa Healthcare, New York, International Journal of Pharmaceutics (2007), 351pp., ISBN:0-8493-9073-7.358.307.10.1016/j.ijpharm.2008.02.006.
2. Merisko-Liversidge E, Liversidge GG, Cooper ER. 2003. Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur J Pharm Sci, 18(2):113-20. doi: 10.1016/s0928-0987(02)00251-8.
3. R. Ravichandran. 2009. Nanoparticles in Drug Delivery: Potential Green Nanobiomedicine Applications. International Journal of Green Nanotechnology: Biomedicine, 1:2, B108-B130, DOI: 10.1080/19430850903430427
4. Keck CM, Müller RH. 2006. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenization. Eur J Pharm Biopharm, 62(1):3-16. doi: 10.1016/j.ejpb.2005.05.009.
5. Shegokar, Ranjita & Müller, Rainer. 2010. Nanocrystals: Industrially feasible multifunctional formulation technology for poorly soluble actives. International journal of pharmaceutics, 399. 129-39. 10.1016/j.ijpharm.2010.07.044.
6. Butler JM, Dressman JB. 2010. The developability classification system: application of biopharmaceutics concepts to formulation development. J Pharm Sci, 99(12):4940-54. doi: 10.1002/jps.22217.
7. Möschwitzer JP. 2013. Drug nanocrystals in the commercial pharmaceutical development process. Int J Pharm, 453(1):142-56. doi: 10.1016/j.ijpharm.2012.09.034.
8. Jog R, Burgess DJ. 2017. Pharmaceutical Amorphous Nanoparticles. J Pharm Sci, 106(1):39-65. doi: 10.1016/j.xphs.2016.09.014.
9. Tran TT, Tran PH, Nguyen MN, Tran KT, Pham MN, Tran PC, Vo TV. 2014. Amorphous isradipine nanosuspension by the sonoprecipitation method. Int J Pharm, 474(1-2):146-50. doi: 10.1016/j.ijpharm.2014.08.017.
10. Xia D, Quan P, Piao H, Piao H, Sun S, Yin Y, Cui F. 2010. Preparation of stable nitrendipine nanosuspensions using the precipitation-ultrasonication method for enhancement of dissolution and oral bioavailability. Eur J Pharm Sci, 40(4):325-34. doi: 10.1016/j.ejps.2010.04.006.
11. el-batal, Ahmed & Elmenshawi, Shahira & Ali, Ahmed & Eldbaiky, Enas. 2018. Preparation and Characterization of Silymarin Nanocrystals and Phytosomes with Investigation of their Stability using Gamma Irradiation. Indian Journal of Pharmaceutical Education and Research, 52. S174-S183. 10.5530/ijper.52.4s.96.
12. Gülsün İnal, Tuğba & Gürsoy, R. Neslihan & Oner, Levent. 2009. Nanocrystal Technology For Oral Delivery of Poorly Water-Soluble Drugs. Fabad Journal of Pharmaceutical Sciences, 34.
13. Patravale, Vandana & Date, Abhijit & Kulkarni, R. 2004. Nanosuspensions: A promising drug delivery strategy. The Journal of pharmacy and pharmacology, 56. 827-40. 10.1211/0022357023691.
14. Eerdenbrugh, Bernard & Van den Mooter, Guy & Augustijns, Patrick. 2008. Top-Down Production of Drug Nanocrystals - Nanosuspension Stabilization, Miniaturization and Transformation In to Solid Products. International journal of pharmaceutics, 364. 64-75. 10.1016/j.ijpharm.2008.07.023.
15. Jinsong Tao, Shing Fung Chow, Ying Zheng. 2019. Application of flash nanoprecipitation to fabricate poorly water-soluble drug nanoparticles, Acta Pharmaceutica Sinica B, Volume 9 Issue 1, Pages 4-18 ISSN 2211-3835 https://doi.org/10.1016/j.apsb.2018.11.001
16. Möschwitzer JP. 2013. Drug nanocrystals in the commercial pharmaceutical development process. Int J Pharm, 453(1):142-56. doi: 10.1016/j.ijpharm.2012.09.034.
17. https://www.researchgate.net/figure/Fig-2-Methods-for-manufacturing-nanocrystals-along-with-characterization-techniques_fig2_341337334]
18. Malamatari M, Taylor KMG, Malamataris S, Douroumis D, Kachrimanis K. 2018. Pharmaceutical nanocrystals: production by wet milling and applications. Drug Discov Today, 23(3):534-547. doi: 10.1016/j.drudis.2018.01.016.
19. Shankar, s. j., b. h., . j. g., r. s., a., metikurki, b., & rehamathulla, m. 2020. A review on the role of nanocrystals and nanosuspensions in drug delivery systems. International Journal of Applied Pharmaceutics, 12(1), 10–16. https://doi.org/10.22159/ijap.2020v12i1.35508
20. Wang H, Xiao Y, Wang H, Sang Z, Han X, Ren S, Du R, Shi X, Xie Y.2019. Development of daidzein nanosuspensions: Preparation, characterization, in vitro evaluation, and pharmacokinetic analysis. Int J Pharm, 566:67-76. doi: 10.1016/j.ijpharm.2019.05.051.
21. Karakucuk A, Teksin ZS, Eroglu H, Celebi N. 2019. Evaluation of improved oral bioavailability of ritonavir nanosuspension. Eur J Pharm Sci, 131:153-158. doi: 10.1016/j.ejps.2019.02.028.
22. P, Nagaraju & K, Krishnachaithanya & V.D.N, Srinivas & S.V.N, Padma. 2010. Nanosuspensions: A Promising Drug Delivery Systems. International Journal of Pharmaceutical Sciences and Nanotechnology, 2. 679-684. 10.37285/ijpsn.2009.2.4.1.
23. Sumathi, R. & Tamizharasi, S. & Sivakumar, T. 2017. Formulation and evaluation of polymeric nanosuspension of naringenin. International Journal of Applied Pharmaceutics, 9. 60. 10.22159/ijap.2017v9i6.21674.
24. M.J. Chen, H.-W. Hui, T. Lee, P. Kurtulik, S. Surapaneni. 2015. Nano-suspension of a poorly soluble drug via micro fluidization process, Google Patents,
25. R. Müller, C. Jacobs, O. Kayser. 2002. Disso Cubes: A novel formulation for poorly soluble and poorly bioavailable drugs, Rathbone M, Hadgraft J, Roberts M. Modified-Release Drug Delivery Technology. Informa Healthcare, 135-149.
26. S. Katteboinaa, V. Chandrasekhar, S. Balaji. 2009. Drug nanocrystals: A novel formulation approach for poorly soluble drugs, International journal of pharmtech research, 682-694.
27. Kreuter J. 2001. Nanoparticulate systems for brain delivery of drugs. Adv Drug Deliv Rev, 47(1):65-81. doi: 10.1016/s0169-409x(00)00122-8. PMID: 11251246.
28. Zhou, Y., Du, J., Wang, L. et al.2016. State of the art of nanocrystals technology for delivery of poorly soluble drugs. J Nanopart Res 18, https://doi.org/10.1007/s11051-016-3575-y

29. R.P. BRUNO, R. MCILWRICK. 2001. Microfluidizer processor technology for high performance particle size reduction, mixing and dispersion, Paperback APV, 77-89.
30. de Waard H, Frijlink HW, Hinrichs WL. 2011. Bottom-up preparation techniques for nanocrystals of lipophilic drugs. Pharm Res, 28(5):1220-3. doi: 10.1007/s11095-010-0323-3.
31. J.E. Kipp, J.C.T. Wong, M.J. Doty, C.L. 2003. Rebbeck, Microprecipitation method for preparing submicron suspensions, Google Patents,
32. R. Müller, J. Möschwitzer. 2005. Method and apparatus for the production of ultrafine particles and coating of such particles, 053
33. Chen Z, Wu W, Lu Y. 2020. What is the future for nanocrystal-based drug-delivery systems? Ther Deliv, 11(4):225-229. doi: 10.4155/tde-2020-0016.
34. Bott S, Hart W. 1990. Particle size analysis utilizing polarization intensity differential scattering. U.S. Patent 4 (953),978.
35. Shegokar R, Müller RH. 2010. Nanocrystals: industrially feasible multifunctional formulation technology for poorly soluble actives. Int J Pharm, 399(1-2):129-39. doi: 10.1016/j.ijpharm.2010.07.044.
36. Müller RH, Gohla S, Keck CM. 2011.State of the art of nanocrystals--special features, production, nanotoxicology aspects and intracellular delivery. Eur J Pharm Biopharm, 78(1):1-9. doi: 10.1016/j.ejpb.2011.01.007.
37. Aziz, Tariq & Fan, Hong & Zhang, · & Haq, Fazal & Ullah, Asmat & Ullah, Roh & Farman, Farman & Khan, Farman & Iqbal, Mudassir. 2020. Advance Study of Cellulose Nanocrystals Properties and Applications. Journal of Polymers and the Environment, 10.1007/s10924-020-01674-2.
38. Sawant KK, Patel MH, Patel K. 2016. Cefdinir nanosuspension for improved oral bioavailability by media milling technique: formulation, characterization and in vitro-in vivo evaluations. Drug Dev Ind Pharm, 42(5):758-68. doi: 10.3109/03639045.2015.1104344.
39. Dressman JB, Amidon GL, Reppas C, Shah VP. 1998. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharm Res, 15(1):11-22. doi: 10.1023/a:1011984216775.
40. Sawant KK, Patel MH, Patel K. 2016. Cefdinir nanosuspension for improved oral bioavailability by media milling technique: formulation, characterization and in vitro-in vivo evaluations. Drug Dev Ind Pharm, 42(5):758-68. doi: 10.3109/03639045.2015.1104344.
41. Mitra Mosharraf, Christer Nyström. 1995. The effect of particle size and shape on the surface specific dissolution rate of microsized practically insoluble drugs, International Journal of Pharmaceutics, Volume 122, Issues 1–2, 1995, Pages 35-47, ISSN 0378-5173, https://doi.org/10.1016/0378-5173(95)00033-F.
42. Im SH, Jung HT, Ho MJ, Lee JE, Kim HT, Kim DY, Lee HC, Choi YS, Kang MJ. 2019. Montelukast Nanocrystals for Transdermal Delivery with Improved Chemical Stability. Pharmaceutics, 12(1):18. doi: 10.3390/pharmaceutics12010018.
43. Müller RH, Jacobs C. 2002. Buparvaquone mucoadhesive nanosuspension: preparation, optimization and long-term stability. Int J Pharm, 237(1-2):151-61. doi: 10.1016/s0378-5173(02)00040-6.
44. Jacobs C, Müller RH. 2002. Production and characterization of a budesonide nanosuspension for pulmonary administration. Pharm Res, 19(2):189-94. doi: 10.1023/a:1014276917363.
45. Lee J, Lee SJ, Choi JY, Yoo JY, Ahn CH. 2005. Amphiphilic amino acid copolymers as stabilizers for the preparation of nanocrystal dispersion. Eur J Pharm Sci, 24(5):441-9. doi: 10.1016/j.ejps.2004.12.010.
46. Kipp JE. 2004.The role of solid nanoparticle technology in the parenteral delivery of poorly water-soluble drugs. Int J Pharm, 284(1-2):109-22. doi: 10.1016/j.ijpharm.2004.07.019.
47. Mantzaris, Nikos. 2005. Liquid-phase synthesis of nanoparticles: Particle size distribution dynamics and control. Chemical Engineering Science, 60. 4749-4770. 10.1016/j.ces.2005.04.012.
48. Mantzaris, Nikos. 2005. Liquid-phase synthesis of nanoparticles: Particle size distribution dynamics and control. Chemical Engineering Science, 60. 4749-4770. 10.1016/j.ces.2005.04.012.
49. Deng J, Huang L, Liu F. 2010. Understanding the structure and stability of paclitaxel nanocrystals. Int J Pharm, 390(2):242-9. doi: 10.1016/j.ijpharm.2010.02.013.
50. Gigliobianco MR, Casadidio C, Censi R, Di Martino P. 2018. Nanocrystals of Poorly Soluble Drugs: Drug Bioavailability and Physicochemical Stability. Pharmaceutics, 10(3):134. doi: 10.3390/pharmaceutics10030134.
51. Raja SN, Bekenstein Y, Koc MA, Fischer S, Zhang D, Lin L, Ritchie RO, Yang P, Alivisatos AP. 2016. Encapsulation of Perovskite Nanocrystals into Macroscale Polymer Matrices: Enhanced Stability and Polarization. ACS Appl Mater Interfaces, 8(51):35523-35533. doi: 10.1021/acsami.6b09443.
52. Chogale MM, Ghodake VN, Patravale VB. 2016. Performance Parameters and Characterizations of Nanocrystals: A Brief Review. Pharmaceutics, 8(3):26. doi: 10.3390/pharmaceutics8030026.
53. Dominique Duchêne, Gilles Ponchel. 1997. Bioadhesion of solid oral dosage forms, why and how?, European Journal of Pharmaceutics and Biopharmaceutics, Volume 44, Issue 1, Pages 15-23, ISSN 0939-6411, https://doi.org/10.1016/S0939-6411(97)00097-0.
54. Castañeda, Luis. 2019. A Facile Method for Formulation of Atenolol Nanocrystal Drug with Enhanced Bioavailability. 10.5772/intechopen.88191.
55. Dominique Duchêne, Gilles Ponchel. 1997. Bioadhesion of solid oral dosage forms, why and how?, European Journal of Pharmaceutics and Biopharmaceutics, Volume 44, Issue 1, Pages 15-23, ISSN 0939-6411, https://doi.org/10.1016/S0939-6411(97)00097-0.
56. Castañeda, Luis. 2019. A Facile Method for Formulation of Atenolol Nanocrystal Drug with Enhanced Bioavailability. 10.5772/intechopen.88191.

57. Dominique Duchêne, Gilles Ponchel.1997. Bioadhesion of solid oral dosage forms, why and how?, European Journal of Pharmaceutics and Biopharmaceutics, Volume 44, Issue 1, Pages 15-23, ISSN 0939-6411, https://doi.org/10.1016/S0939-6411(97)00097-0.
58. Gao L, Liu G, Ma J, Wang X, Zhou L, Li X. 2012. Drug nanocrystals: In vivo performances. J Control Release, 160(3):418-30. doi: 10.1016/j.jconrel.2012.03.013.
59. Kayser O, Olbrich C, Yardley V, Kiderlen AF, Croft SL. 2003. Formulation of amphotericin B as nanosuspension for oral administration. Int J Pharm, 254(1):73-5. doi: 10.1016/s0378-5173(02)00686-5.
60. Mauludin R, Müller RH, Keck CM. 2009. Development of an oral rutin nanocrystal formulation. Int J Pharm, 370(1-2):202-9. doi: 10.1016/j.ijpharm.2008.11.029.
61. Mitri K, Shegokar R, Gohla S, Anselmi C, Müller RH. 2011. Lutein nanocrystals as antioxidant formulation for oral and dermal delivery. Int J Pharm, 420(1):141-6. doi: 10.1016/j.ijpharm.2011.08.026.
62. Zhang J, Lv H, Jiang K, Gao Y. 2011. Enhanced bioavailability after oral and pulmonary administration of baicalein nanocrystal. Int J Pharm, 420(1):180-8. doi: 10.1016/j.ijpharm.2011.08.023.
63. Ige PP, Baria RK, Gattani SG. 2013. Fabrication of fenofibrate nanocrystals by probe sonication method for enhancement of dissolution rate and oral bioavailability. Colloids Surf B Biointerfaces, 108:366-73. doi: 10.1016/j.colsurfb.2013.02.043.
64. Müller RH, Jacobs C. 2002. Buparvaquone mucoadhesive nanosuspension: preparation, optimization and long-term stability. Int J Pharm, 237(1-2):151-61. doi: 10.1016/s0378-5173(02)00040-6.
65. Li W, Yang Y, Tian Y, Xu X, Chen Y, Mu L, Zhang Y, Fang L. 2011. Preparation and in vitro/in vivo evaluation of revaprazan hydrochloride nanosuspension. Int J Pharm, 408(1-2):157-62. doi: 10.1016/j.ijpharm.2011.01.059.
66. Möschwitzer J, Achleitner G, Pomper H, Müller RH. 2004. Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology. Eur J Pharm Biopharm, 58(3):615-9. doi: 10.1016/j.ejpb.2004.03.022.
67. Ganta S, Paxton JW, Baguley BC, Garg S. 2009. Formulation and pharmacokinetic evaluation of an asulacrine nanocrystalline suspension for intravenous delivery. Int J Pharm, 367(1-2):179-86. doi: 10.1016/j.ijpharm.2008.09.022.
68. Pawar VK, Singh Y, Meher JG, Gupta S, Chourasia MK. 2014. Engineered nanocrystal technology: in-vivo fate, targeting and applications in drug delivery. J Control Release, 183:51-66. doi: 10.1016/j.jconrel.2014.03.030.
69. Jinno J, Kamada N, Miyake M, Yamada K, Mukai T, Odomi M, Toguchi H, Liversidge GG, Higaki K, Kimura T. 2006. Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs. J Control Release, 111(1-2):56-64. doi: 10.1016/j.jconrel.2005.11.013.
70. Merisko-Liversidge E, Liversidge GG, Cooper ER. 2003. Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur J Pharm Sci, 18(2):113-20. doi: 10.1016/s0928-0987(02)00251-8.
71. Peters K, Leitzke S, Diederichs JE, Borner K, Hahn H, Müller RH, Ehlers S. 2000. Preparation of a clofazimine nanosuspension for intravenous use and evaluation of its therapeutic efficacy in murine Mycobacterium avium infection. J Antimicrob Chemother, 45(1):77-83. doi: 10.1093/jac/45.1.77.
72. Hu L, Kong D, Hu Q, Gao N, Pang S. 2015. Evaluation of High-Performance Curcumin Nanocrystals for Pulmonary Drug Delivery Both In Vitro and In Vivo. Nanoscale, 10(1):381. doi: 10.1186/s11671-015-1085-y.
73. Hu X, Yang FF, Wei XL, Yao GY, Liu CY, Zheng Y, Liao YH. 2017. Curcumin Acetate Nanocrystals for sustained Pulmonary Delivery: Preparation, Characterization and In Vivo Evaluation. J Biomed Nanotechnol, 13(1):99-09. doi: 10.1166/jbn.2017.2326.
74. Ostrander KD, Bosch HW, Bondanza DM. 1999. An in-vitro assessment of a NanoCrystal beclomethasone dipropionate colloidal dispersion via ultrasonic nebulization. Eur J Pharm Biopharm, 48(3):207-15. doi: 10.1016/s0939-6411(99)00049-1.
75. Zhang J, Lv H, Jiang K, Gao Y. 2011. Enhanced bioavailability after oral and pulmonary administration of baicalein nanocrystal. Int J Pharm, 420(1):180-8. doi: 10.1016/j.ijpharm.2011.08.023.
76. Jacobs C, Müller RH. 2002. Production and characterization of a budesonide nanosuspension for pulmonary administration. Pharm Res, 19(2):189-94. doi: 10.1023/a:1014276917363.
77. Thakur RR, Kashiv M. 2011. Modern delivery systems for ocular drug formulations: a comparative overview WRT conventional dosage form. Int J Res Pharmaceut Biomed Sci, 2:8–18.
78. Tangri, Pranshu & Khurana, Saanidhya. 2011. Basics of ocular drug delivery systems. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2. 1541-1552.
79. Shafaie S, Hutter V, Cook MT, Brown MB, Chau DY. 2016. In Vitro Cell Models for Ophthalmic Drug Development Applications. Biores Open Access, 5(1):94-108. doi: 10.1089/biores.2016.0008.
80. Kassem MA, Abdel Rahman AA, Ghorab MM, Ahmed MB, Khalil RM. 2007. Nanosuspension as an ophthalmic delivery system for certain glucocorticoid drugs. Int J Pharm, 340(1-2):126-33. doi: 10.1016/j.ijpharm.2007.03.011.
81. Strebhardt K, Ullrich A. 2008. Paul Ehrlich's magic bullet concept: 100 years of progress. Nat Rev Cancer, 8(6):473-80. doi: 10.1038/nrc2394.
82. Lu Y, Li Y, Wu W. 2016. Injected nanocrystals for targeted drug delivery. Acta Pharm Sin B, 6(2):106-13. doi: 10.1016/j.apsb.2015.11.005.
83. Shegokar R, Müller RH. 2010. Nanocrystals: industrially feasible multifunctional formulation technology for poorly soluble actives. Int J Pharm, 399(1-2):129-39. doi: 10.1016/j.ijpharm.2010.07.044.
84. Chai Z, Ran D, Lu L, Zhan C, Ruan H, Hu X, Xie C, Jiang K, Li J, Zhou J, Wang J, Zhang Y, Fang RH, Zhang L, Lu W. 2019. Ligand-Modified Cell Membrane Enables the Targeted Delivery of Drug Nanocrystals to Glioma. ACS Nano, 13(5):5591-5601. doi: 10.1021/acsnano.9b00661.

85. Muheem A, Shakeel F, Warsi MH, Jain GK, Ahmad FJ. 2017. A Combinatorial Statistical Design Approach to Optimize the Nanostructured Cubosomal Carrier System for Oral Delivery of Ubidecarenone for Management of Doxorubicin-Induced Cardiotoxicity: In Vitro-In Vivo Investigations. J Pharm Sci, 106(10):3050-3065. doi: 10.1016/j.xphs.2017.05.026.
86. Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S, Monteiro-Riviere N, Warheit D, Yang H. 2005. ILSI Research Foundation/Risk Science Institute Nanomaterial Toxicity Screening Working Group. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol, 2:8. doi: 10.1186/1743-8977-2-8.
87. Manzanares D, Ceña V. 2020. Endocytosis: The Nanoparticle and Submicron Nanocompounds Gateway into the Cell. Pharmaceutics, 12(4):371. doi: 10.3390/pharmaceutics12040371.
88. Oh N, Park JH. 2014. Endocytosis and exocytosis of nanoparticles in mammalian cells. Int J Nanomedicine, 9 (Suppl 1):51-63. doi: 10.2147/IJN.S26592.
89. Varaporn Buraphacheep Junyaprasert, Boontida Morakul. 2015. Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs, Asian Journal of Pharmaceutical Sciences, Volume 10, Issue 1, Pages 13-23, ISSN 1818-0876, https://doi.org/10.1016/j.ajps.2014.08.005.
90. P.J. Haines, M. Reading, F.W. Wilburn. 1998. Chapter 5 - Differential Thermal Analysis and Differential Scanning Calorimetry, Editor(s): Michael E. Brown, Handbook of Thermal Analysis and Calorimetry, Elsevier Science B.V., Volume 1, Pages 279-361, ISSN 1573-4374, ISBN 9780444820853, https://doi.org/10.1016/S1573-4374(98)80008-3.
91. P.J. Haines, M. Reading, F.W. Wilburn. 1998. Chapter 5 - Differential Thermal Analysis and Differential Scanning Calorimetry, Editor(s): Michael E. Brown, Handbook of Thermal Analysis and Calorimetry, Elsevier Science B.V., Volume 1, Pages 279-361, ISSN 1573-4374, ISBN 9780444820853, https://doi.org/10.1016/S1573-4374(98)80008-3.
92. Robert L Danley. 2002. New heat flux DSC measurement technique, Thermochimica Acta, Volume 395, Issues 1–2, Pages 201-208, ISSN 0040-6031, https://doi.org/10.1016/S0040-6031(02)00212-5.
93. N. Zucca, G. Erriu, S. Onnis, A. Longoni. 2004. An analytical expression of the output of a power-compensated DSC in a wide temperature range, Thermochimica Acta, Volume 413, Issues 1–2, Pages 117-125, ISSN 0040-6031, https://doi.org/10.1016/j.tca.2003.10.006
94. Robert L Danley. 2002. New heat flux DSC measurement technique, Thermochimica Acta, Volume 395, Issues 1–2, Pages 201-208, ISSN 0040-6031, https://doi.org/10.1016/S0040-6031(02)00212-5.
95. P.J. Haines, M. Reading, F.W. Wilburn. 1998. Chapter 5 - Differential Thermal Analysis and Differential Scanning Calorimetry, Editor(s): Michael E. Brown, Handbook of Thermal Analysis and Calorimetry, Elsevier Science B.V., Volume 1, Pages 279-361, ISSN 1573-4374, ISBN 9780444820853, https://doi.org/10.1016/S1573-4374(98)80008-3
96. N. Zucca, G. Erriu, S. Onnis, A. Longoni. 2004.An analytical expression of the output of a power-compensated DSC in a wide temperature range, Thermochimica Acta, Volume 413, Issues 1–2, Pages 117-125, ISSN 0040-6031, https://doi.org/10.1016/j.tca.2003.10.006.
97. P. Kocbek, S. Baumgartner, J. Kristl. 2006. Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs, International Journal of Pharmaceutics, Volume 312, Issues 1–2, Pages 179-186, ISSN 0378-5173, https://doi.org/10.1016/j.ijpharm.2006.01.008.
98. Shawn X. Yin, Miriam Franchini, Jinling Chen, Alice Hsieh, Sandy Jen, Tu Lee, Munir Hussain, Ronald Smith. 2005. Bioavailability enhancement of a COX-2 inhibitor, BMS-347070, from a nanocrystalline dispersion prepared by spray-drying, Journal of Pharmaceutical Sciences, Volume 94, Issue 7, Pages 1598-1607, ISSN 0022-3549, https://doi.org/10.1002/jps.20366.
99. Huang, Y., Luo, X., You, X. et al. 2013. The Preparation and Evaluation of Water-Soluble SKLB610 Nanosuspensions with Improved Bioavailability. AAPS PharmSciTech 14, 1236–1243. https://doi.org/10.1208/s12249-013-0005-7.
100. Koneti Venkata Mahesh, Sachin Kumar Singh, Monica Gulati. 2014. A comparative study of top-down and bottom-up approaches for the preparation of nanosuspensions of glipizide, Powder Technology, Volume 256, Pages 436-449, ISSN 0032-5910, https://doi.org/10.1016/j.powtec.2014.02.011.
101. Hu, L., Kong, D., Hu, Q. et al. 2015. Evaluation of High-Performance Curcumin Nanocrystals for Pulmonary Drug Delivery Both In Vitro and In Vivo. Nanoscale Res Lett **10**, 381. https://doi.org/10.1186/s11671-015-1085-y
102. de Waard H, De Beer T, Hinrichs WL, Vervaet C, Remon JP, Frijlink HW. 2010. Controlled crystallization of the lipophilic drug fenofibrate during freeze-drying: elucidation of the mechanism by in-line Raman spectroscopy. AAPS J, 12(4):569-75. doi: 10.1208/s12248-010-9215-z.
103. Ali HS, York P, Ali AM, Blagden N. 2011. Hydrocortisone nanosuspensions for ophthalmic delivery: A comparative study between microfluidic nanoprecipitation and wet milling. J Control Release, 149(2):175-81. doi: 10.1016/j.jconrel.2010.10.007.
104. Pireddu R, Sinico C, Ennas G, Marongiu F, Muzzalupo R, Lai F, Fadda AM. 2015. Novel nanosized formulations of two diclofenac acid polymorphs to improve topical bioavailability. Eur J Pharm Sci, 77:208-15. doi: 10.1016/j.ejps.2015.06.006.
105. Keck C, Müller R. 2005. Particle size analysis with laser diffractometry is not sensitive enough to detect changes in a lipid carrier system. In Proceedings of the Annual Meeting of the American Association of Pharmaceutical Scientists (AAPS), Nashville, TN, USA (pp. 6-10).
106. Keck C, Müller R. 2005. Particle size analysis with laser diffractometry is not sensitive enough to detect changes in a lipid carrier system. InProceedings of the Annual Meeting of the American Association of Pharmaceutical Scientists (AAPS), Nashville, TN, USA (pp. 6-10).
107. Bott SE, Hart WH, inventors. 1990. Coulter Electronics of New England Inc, assignee. Particle size analysis utilizing polarization intensity differential scattering. United States patent US 4,953,978.
108. Xu R, inventor. 2005. Coulter International Corp, assignee. Extracted polarization intensity differential scattering for particle characterization. United States patent US 6,859,276.

109. Keck C, Müller R. 2005. Particle size analysis with laser diffractometry is not sensitive enough to detect changes in a lipid carrier system. In Proceedings of the Annual Meeting of the American Association of Pharmaceutical Scientists (AAPS), Nashville, TN, USA (pp. 6-10).
110. Gao, L., Zhang, D. & Chen, M. 2008. Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system. J Nanopart Res 10, 845–862. https://doi.org/10.1007/s11051-008-9357-4
111. Rawle, A. 2004. Nano powders—An oxymoron? In Proceedings of the Particles 2004—Particle Synthesis, Characterization, and Particle-Based Advanced Materials, Orlando, FL, USA, 6–9
112. Gao, L., Zhang, D. & Chen, M. 2008. Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system. J Nanopart Res 10, 845–862. https://doi.org/10.1007/s11051-008-9357-4
113. Rawle A. 2004. Nanopowders—An oxymoron? In Proceedings of the Particles 2004—Particle Synthesis, Characterization, and Particle-Based Advanced Materials, Orlando, FL, USA, 6–9 .36.
114. Moribe K, Wanawongthai C, Shudo J, Higashi K, Yamamoto K. 2008. Morphology and surface States of colloidal probucol nanoparticles evaluated by atomic force microscopy. Chem Pharm Bull (Tokyo), 56(6):878-80. doi: 10.1248/cpb.56.878.
115. Liu P, Viitala T, Kartal-Hodzic A, Liang H, Laaksonen T, Hirvonen J, Peltonen L. 2015. Interaction studies between indomethacin nanocrystals and PEO/PPO copolymer stabilizers. Pharm Res, 32(2):628-39. doi: 10.1007/s11095-014-1491-3.
116. Rawle A. 2004. Nanopowders—An oxymoron? In Proceedings of the Particles 2004—Particle Synthesis, Characterization, and Particle-Based Advanced Materials, Orlando, FL, USA, 36.
117. Moribe K, Wanawongthai C, Shudo J, Higashi K, Yamamoto K. 2008. Morphology and surface States of colloidal probucol nanoparticles evaluated by atomic force microscopy. Chem Pharm Bull (Tokyo), 56(6):878-80. doi: 10.1248/cpb.56.878.
118. Liu P, Viitala T, Kartal-Hodzic A, Liang H, Laaksonen T, Hirvonen J, Peltonen L. 2015. Interaction studies between indomethacin nanocrystals and PEO/PPO copolymer stabilizers. Pharm Res, 32(2):628-39. doi: 10.1007/s11095-014-1491-3.
119. Li Y, Dong L, Jia A, Chang X, Xue H. 2006. Preparation and characterization of solid lipid nanoparticles loaded traditional Chinese medicine. Int J Biol Macromol, 38(3-5):296-9. doi: 10.1016/j.ijbiomac.2006.03.006.
120. Ige PP, Baria RK, Gattani SG. 2013. Fabrication of fenofibrate nanocrystals by probe sonication method for enhancement of dissolution rate and oral bioavailability. Colloids Surf B Biointerfaces, 108:366-73. doi: 10.1016/j.colsurfb.2013.02.043.
121. Sarnes A, Østergaard J, Jensen SS, Aaltonen J, Rantanen J, Hirvonen J, Peltonen L. 2013. Dissolution study of nanocrystal powders of a poorly soluble drug by UV imaging and channel flow methods. Eur J Pharm Sci, 50(3-4):511-9. doi: 10.1016/j.ejps.2013.08.030.
122. Frank KJ, Westedt U, Rosenblatt KM, Hölig P, Rosenberg J, Mägerlein M, Fricker G, Brandl M. 2014. What is the mechanism behind increased permeation rate of a poorly soluble drug from aqueous dispersions of an amorphous solid dispersion? J Pharm Sci, 103(6):1779-86. doi: 10.1002/jps.23979.
123. Liu P, Viitala T, Kartal-Hodzic A, Liang H, Laaksonen T, Hirvonen J, Peltonen L. 2015. Interaction studies between indomethacin nanocrystals and PEO/PPO copolymer stabilizers. Pharm Res, 32(2):628-39. doi: 10.1007/s11095-014-1491-3.
124. Patzelt A, Richter H, Knorr F, Schäfer U, Lehr CM, Dähne L, Sterry W, Lademann J. 2011. Selective follicular targeting by modification of the particle sizes. J Control Release, 150(1):45-8. doi: 10.1016/j.jconrel.2010.11.015.
125. Lademann J, Richter H, Teichmann A, Otberg N, Blume-Peytavi U, Luengo J, Weiss B, Schaefer UF, Lehr CM, Wepf R, Sterry W. 2007. Nanoparticles--an efficient carrier for drug delivery into the hair follicles. Eur J Pharm Biopharm, 66(2):159-64. doi: 10.1016/j.ejpb.2006.10.019.
126. Lang J, Roehrs R, Jani R. 2006. Ophtalmic Preparations. In Remington: The Science and Practice of Pharmacy; Lippincott Williams & Wilkins: Philadelphia, PA, USA,
127. Wei Li, Peng Quan, Yaqiong Zhang, Jing Cheng, Jie Liu, Dongmei Cun, Rongwu Xiang, Liang Fang. 2014. Influence of drug physicochemical properties on absorption of water insoluble drug nanosuspensions, International Journal of Pharmaceutics, Volume 460, Issues 1–2, Pages 13-23, ISSN 0378-5173, https://doi.org/10.1016/j.ijpharm.2013.10.038.
128. Guo Y, Luo J, Tan S, Otieno BO, Zhang Z. 2013. The applications of Vitamin E TPGS in drug delivery. Eur J Pharm Sci, 49(2):175-86. doi: 10.1016/j.ejps.2013.02.006.
129. Chen Y, Li T. 2015. Cellular Uptake Mechanism of Paclitaxel Nanocrystals Determined by Confocal Imaging and Kinetic Measurement. AAPS J, 17(5):1126-34. doi: 10.1208/s12248-015-9774-0.
130. Jarvis M, Krishnan V, Mitragotri S. 2018. Nanocrystals: A perspective on translational research and clinical studies. Bioeng Transl Med, 4(1):5-16. doi: 10.1002/btm2.10122.