# Advances in Nano crystal: A Detailed Overview of Methodologies, Types, Applications, Characterization and Marketed Products of Nano crystals.

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## Abstract

Nearly 40% of the recently identified therapeutic moieties have limited clinical usefulness due to poor bioavailability, which is linked to their poor solubility. Alternative formulation solutions for such medications must be investigated due to their low solubility, high log p value, high melting point, and high dose. The formulation of the medications as "Nano-crystals" is one such innovative strategy. Drugs and surfactants/stabilizers make up the majority of Nano-crystals, which are produced using either "top-down" or "bottom-up" techniques. By increasing the drug's bioavailability, reducing the dosage needed, and promoting prolonged release, Nano-crystals contribute to the therapeutic effectiveness of medications. Particle size, saturation solubility, and dissolving velocity are some of the properties of Nano-crystals that affect their enhanced performance and are necessary for this effect. To assess these qualities, a number of advanced methods have been created. Along with a brief overview about Nano-crystals, this article provides a detailed description of the various making methodologies.

**Keywords:** Nano-crystals, particle size, particle shape, top-down techniques, bottom-up techniques.

**Introduction:** Formulating poorly water-soluble pharmacological substances is currently the most tedious challenge for formulation scientists. The development of high throughput screening techniques is primarily accountable for the increased number of recently identified medications with low water solubility. Literature data indicate that about 40% of medications entering the formulation research are poorly soluble in water. It is important to interpret the official term "poorly soluble drugs" broadly, meaning that they are either incompatible with aqueous or oily systems, or they are only weakly soluble in hydrophilic or lipophilic solvents. (1, 2) Drugs with weak water solubility have poor bioavailability, which impacts their therapeutic effectiveness. In an effort to increase these medication's bioavailability, methods to make them more soluble in water are being investigated. Numerous methods have been studied in this effort to improve bioavailability. The majority of these techniques, however, are limited to medications with a particular chemistry or a specified molecular size, shape, or conformation. (1) The creation of Nano-scale drug formulations has assisted in resolving the bioavailability issues associated with certain poorly water-soluble BCS Class 2 and Class 4 medications. One type of these Nano-sized formulations are called "Nano-crystals," which are composed of drug particles at the Nano-scale that have been stabilized by an appropriate stabilizer or surfactant. Crystals in the Nano-meters range, typically between a few Nano-meters and 1000 nm, are known as drug Nano-crystals. The term "Nano suspensions" refers to drug Nano-crystals that are dispersed in an aqueous medium. Another feature of the Nano-crystals is that, unlike polymeric or lipidic Nanoparticles, they are entirely made of the medication and do not contain any carrier material. They are often made by allowing the drug to precipitate out of its organic solvent after adding an aqueous solution of a surfactant or stabilizer, or by undergoing rigorous particle size reduction techniques on the drug's macrosized dispersion while a surfactant or stabilizer is present. (1)

## Nano-crystals: Briefing

Crystals smaller than 1 μm are called Nano-crystals. When a crystal's particle size is reduced to around 100 nm, the material's characteristics drastically alter. Drug's surface area and solubility are increased by their reduced size, and the bioavailability of poorly soluble medications/drugs rises proportionately. Compared to micro-ionization, Nano-iozation has an additional effect. In addition to increasing the surface area, it also raises the saturation solubility. Ordinarily sized particles' solubility is a compound-specific constant that solely depends on solvent and temperature. However, the saturation solubility is also a function of particle size when the crystal's particle size is smaller than 1-2 μm. The particles' strong curvature causes the dissolution pressure to rise, which increases the saturation solubility. (3) The bioavailability of various drugs has been found to increase significantly when administered in the form of Nano-crystals. Eg. This is especially beneficial for drugs where a quick action is desired, such as naproxen for headache relief. Liversidge and Conzentino 1995 (4) conducted a study on the bioavailability of naproxen when naproxen was administered as a conventional tablet, a suspension, and a Nano-suspension. (4) The results showed that the area under the curve (AUC) of blood levels for the analgesic naproxen was 32.7 mgh/l for conventional tablets, 44.7 mgh/l for Naprosyn suspension, and the AUC to 79.5 mgh/l for naproxen administered as Nano-suspensions. Another study examined the effects of administering the gonadotropin inhibitor danazol as a macro-suspension and as a Nano-suspension. When danazol was administered as a micro-emulsion, the relative bioavailability was 5.1%. On the side, Danazol Nano-suspension's bioavailability was discovered to be 82.3%4. Because of their small size, Nano-suspensions have a 100% bioavailability when injected intravenously. Therefore, Nano-crystal technology can be used to make any drug 100% bioavailable. Because of the increased contact area for van der Waals attraction, Nano-crystals can exhibit strong adhesion. After oral administration, the Nanoparticles' adhesion to the gut wall improves absorption and thus bioavailability. (5)

Nano-crystals may be able to reduce the dose to be administered, provide a sustained drug release and increase patient compliance. De Waard et al. (2010) created Nano-crystals of ibuprofen and fenofibrate. He claimed that the structure of the crystals boosts medication absorption significantly to a great extent. Waard argues that administering poorly soluble medications in Nano-crystal form, such as ibuprofen and cholesterol-lowering fenofibrate, can reduce their doses. Increased bioavailability leads to reduced dose frequency, which may enhance patient compliance. (6) Nano-crystals can be put into a variety of dosage forms, allowing them to be administered via many routes. Because of their improved solubility and bioavailability, Nano-crystals can be delivered in patient-friendly oral solid dose forms such as tablets and capsules. Nano-crystals of poorly soluble medications can also be used in cosmetic items to provide great penetration power via dermal application. (3) Drug Nano-crystals are a smart delivery system, a universal principle that can be used to any drug, as any drug may be reduced to Nano-crystals. Furthermore, both lipophilic and hydrophilic medicines can be integrated into Nano-crystals. Another necessary prerequisite for entry into the pharmaceutical business is the availability of large-scale production technologies at a low cost while also meeting regulatory criteria. Nano-crystals technology also meets this requirement. Pearl milling and high pressure homogenization can be used commercially to produce Nano-crystals and are accepted by regulatory authorities. (3)

## Nano-crystals Technology (Methods):

Drug Nano-crystals can be made using either top-down (size reduction by milling or high pressure homogenization) or bottom-up (precipitation methods) approaches. In the former case, solid particles are formed by the association of molecules in the solution; this is a traditional precipitation process. In the latter case, all products are prepared using the top-down technique for industrial production, which is based on the size reduction of relatively large particles into smaller particles by mechanical attrition. (3)

A. Bottom-up technique (Precipitation method)
   A.1. Control Flow Cavitation (CFC)
   A.2. Spray Drying
   A.3. Supercritical Fluid
   A.4. Impinging Jet Crystallization

A.5. Low-Energy Precipitation Method
A.6. Emulsion Method
A.7. Patterned Micro Well and Patterned Gold Islands
A.8. Microfluidics Devices
A.9. Nano-crystal Preparation Using Nanoporous Materials (7)

B. Top down techniques
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B.3. Combination technology
B.3.1. NANOEDGE Technology
B.3.2. SmartCrystal Technology (3)

**A. Bottom-up technique (Precipitation method**) – This method involves dissolving the drug in a solvent, which is then introduced to a non-solvent, causing the finely distributed drug Nano-crystals to precipitate. The precipitation method is easy to use and requires inexpensive tools. For instance, with a high-speed stirrer present, the solvent can be continuously pumped into the non-solvent. The use of static mixers or micro-mixers, which replicate precipitation conditions in a tiny volume (i.e., imitating lab scale settings), is one of the primary strategies. Setting up numerous micro-mixers in simultaneously allows for easy scaling up in the case of micro-mixers. Although this isn't always the case with the micro mixers, this equipment is reasonably inexpensive and straightforward. This method's disadvantage is that the medication must dissolve in at least one solvent. This is a challenge for recently produced medications, though, as they are typically insoluble in both organic and aqueous solutions. Second, at least one non-solvent must be miscible with this solvent. The removal of solvent residues raises the cost of manufacture. When it comes to Nano-crystals, caution must be taken to prevent crystals from enlarging and to maintain their stability at the Nano-scale. The methods that are advised to maintain the particle size in the Nano range are spray drying. Low-Energy Precipitation Method is an additional option to maintain the size of Nano-crystals. At present, there is no pharmaceutical product based on this technology in the market. (3)

A.1. Control Flow Cavitation (CFC) - CFC produces the ideal process conditions for the creation of Nano-crystals, after controlling the size, position, density, and intensity of bubble implosion in the cavitation zone. The particles can be brought to the appropriate particle size distribution by controlling the energy of cavitation and the controlled energy released by the implosion of micro bubbles. (8, 9) In order to create Nano and micro-structured materials, CFC transforms destructive power into constructive force with high intensity energy. Numerous sectors have made use of CFC technology, with various CFC chamber designs tailored for cleaning, chemical, hydrodynamic, and biomedical uses. The CFC is a very effective and scalable technique that has exceptional reproducibility and process control. (7, 9)

A.2. Spray Drying - Spray drying is a one-step method for continuously turning pastes, emulsions, suspensions, slurries, and solutions into powders. Additionally, it makes it possible to produce particles with precise morphological and size characteristics. The typical spray drying method can only produce particles with diameters between 2 and 5 μm due to the restricted collecting efficiency associated with cyclone separators. These drawbacks appear to be addressed by a spray drier that combines a high-efficiency electrostatic powder collection with a piezoelectric-driven vibrating mesh atomizer. Büchi (Switzerland) has created a novel Nano spray dryer technique called B-90, which has been used to accomplish drying and Nano crystallization. (7, 10) The other innovative approach involves dissolving a polymeric dispersion system and a medication in an appropriate solvent. Following spray drying of the resultant solution, the drug-containing powder is either dispersed as submicron particles to form a solid suspension or is the molecularly dispersant system in the polymer matrix to form a solid solution. The spray drying process is incredibly quick, can be customized to any size, may be integrated into a fully automated control system that enables continuous preparation, and comes in a variety of designs. The feedstock can be in the form of a solution, slurry, gel,

suspension, or melt, and the spray dryer can be utilized for both heat-sensitive and heat-resistant products. Because of the present advantages of Nanotechnology, conventional spray dryer systems are under more pressure to generate Nanoparticles with a regulated size distribution and high yield. (10, 11)

A.3. Supercritical Fluid - Recently, methods for producing micro- and Nano-sized particles using supercritical antisolvent (SAS) have been proposed. The three key elements of SAS are the solute, solvent, and supercritical antisolvent. (12) Both the low solubility of the solute in the SAS and the tremendous power of supercritical fluids to dissolve the organic solvents produced a very high degree of supersaturation. High supersaturation and extremely rapid diffusion produce Nanoparticle precipitation that is not achievable by antisolvent precipitation or any other method. The primary benefit is the antisolvent's total elimination through pressure drop to the gas phase. (12) Their diffusivities can be around two orders of magnitude higher than those of liquids, and the solvent power in the case of SAS can be achieved by adjusting the temperature and pressure. Recently, Jarmer et al. employed poly (sebacic unhydride) as a growth inhibitor for griseofulvin using an SAS, while Caputo et al. (12) suggested utilizing SAS to precipitate sulfathiazole from acetone solution by employing urea as a habit modifier. (7, 12)

A.4. Impinging Jet Crystallization - Impinging jet crystallization, a recent development that uses jets to create impinging fluid jet streams and accomplish high intensity micro mixing of the fluids prior to nucleation, is one example of a direct production of small particles. To start the solute's precipitation from solution, the solvent and antisolvent can be micromixed using two or more jets and two fluids with distinct solvent compositions. By combining batch crystallizer running at a regulated constant growth rate with controlled seeding by impinging jet crystallization, Woo et al. investigated the control distribution. Their study's objective was to suggest control solutions that combine optimal control with an impinging jet crystallizer in order to create crystals with a specified crystal size distribution (CSD) Very high energy dissipation rates, effective micromixing, and strictly regulated conditions are provided by impinging jet crystallization, resulting in high-quality products. (13)

A.5. Low-Energy Precipitation Method - It seems that the bottom-up reprecipitation method of creating Nano-crystals is not widely used. Technically speaking, one perceived drawback of the strategy is that it does not work with a large variety of compounds. Through a methodical investigation of three different molecules—ibuprofen, glyburude, and artemsinin—(14) we have demonstrated that basic anti-solvent precipitation may be used to create stable Nano-crystals of uniform size for each of the three medications. Finding suitable stabilizers, which rely on the drug molecule selection, seems to be the technical difficulty. The impacts of temperature, stirring rate, infusion rate, and process factors were also examined. The local super-saturation that occurs during the anti-solvent precipitation process is thought to be influenced by each of these factors. We tried, but failed, to justify the selection of optimal stabilizers in terms of chemical interactions between the stabilizer molecules and crystal surfaces. (14)

A.6. Emulsion Method - The process of creating Nano-crystals using micro-emulsion is becoming increasingly popular in both fundamental and applied industries. The emulsion method of creating organic Nano-crystals involves three steps. The first stage involves creating an emulsion by rapidly adding an organic chemical solution to the aqueous phase at a high temperature. (15)The stable emulsion was created using irradiating ultrasound and a high churning speed. The dispersion is progressively cooled to low temperatures in the second step, which causes the solutes to crystallize. To break the emulsion and extract the organic solvent, an antifoaming agent was used in the third phase. In an aqueous phase, a stable dispersion of Nano-crystals was produced. Ujiiye-Ishii and colleagues (15) explained the emulsion and re-precipitation procedure for creating perylene Nano-crystals. This method's enormous interfacial area, ultralow interfacial tension, and thermodynamic stability of the resultant Nano-crystal dispersions in aqueous mediums are important characteristics. (15)

A.7. Patterned Micro Well and Patterned Gold Islands - The well-defined two-dimensional or limited three-dimensional structures known as patterned micro wells serve primarily as templates to direct the nucleation and development of crystals. Silica, highly ordered graphite, carbon Nanotubes, and self-assembled organic monolayers can all be used as templates. Protein Nano-crystals with uniform morphologies have received a lot of attention lately in an effort to increase bioavailability and provide alternate release routes. According

to Wang et al., patterned micro wells offer a way to regulate protein Nano-crystal crystallization under industry-standard crystallization settings. In the past, organic molecules like glycine were crystallized using patterned Self Assembled Monolayers (SAM). When wetted with polar solvents, the hydrophilic islands in the bifunctional SAMs pattern are surrounded by hydrophobic regions, forming tiny hemispherical droplets. Either slow cooling, slow evaporation, or slow diffusion of an anti-solvent produces the glycine Nano-crystals. Lee et al. demonstrated that the solvent evaporation rate has the biggest impact on the polymorphism result of glycine. (16) Fast evaporation favors β-glycine because it creates high super-saturation, whereas slow evaporation produces α-glycine. Metastable β-glycine (17) polymorphs crystallize as a result of controlling the super-saturation during vapour diffusion studies. According to the study, the metastable β-glycine was obtained by organic vapour diffusion, whilst the α-glycine and γ-glycine polymorphs were prepared by slow cooling and slow evaporation. Based on the island size, Nano-crystals ranging in size from 200 nm to 1.2 μm were created by regulating the initial glycine concentration and the rate of diffusion. Using the Ostwald-Freundlich equation, Kim et al. also determined the solubility of glycine crystals in methanol. They found that 100 nm crystals were twice as soluble as glycine at equilibrium. (16) When the crystal size is less than 97 nm, the action of surface molecules makes β-glycine (17) more stable than α-glycine, according to the solubility values computed from the Ostwald-Freundlich equation for α-glycine and β-glycine polymorphs. With the maximum density of SAMs and a wide range of commercially accessible functional thiols, the patterned SAMs approach is incredibly straightforward. The removal of crystals from the surface is a significant drawback, albeit this can be accomplished via ultrasonography or scraping. (16, 17)

A.8. Microfluidics Devices - The enhanced response control and mixing capabilities of a microfluidic system allow for the continuous creation of Nano-crystals, as the particle size lowers and the distribution of particle sizes becomes more pronounced. The two main types of microfluidic devices are chip-based and capillary-based systems. The basic fluidic components in capillary reactors can be connected by suitable tubing lengths. Chips are usually made using wet etching, micromachining, or a plastic, glass, or silicon substrate and are finely customized. In the synthesis of Nano-crystals, both kinds of reactors are crucial. Two significant types of microfluidic reactors are single-phase and two-phase reactors. (18, 19) Commonly used single phase reactors make it simple to perform multistep reactions and create more complicated structures by injecting miscible streams of chemicals into a channel or capillary where they mix and react. Two significant constraints that can restrict a single phase reactor's performance are the unfavourable velocity dispersion and reactor wall fouling. In two-phase reactors, velocity dispersion is eliminated by injecting an additional immiscible fluid—which can be either a gas or a liquid—into the channel, which splits and forms a split plug that travels through the reactor at a constant speed. By altering the relative hydrostatic pressures at the two outlets, the split plugs' size can be adjusted to match the relative flow rates in the two outlet channels. The conventional reactor for Nano-crystal production, which is unable to meet industrial demand, will not be replaced anytime soon by the microfluidic reactor. (18, 19)

A.9. Nano-crystal Preparation Using Nano-porous Materials – The easiest method for preparing Nano-crystals is a revolutionary procedure that creates Nano-crystals of active medicinal substances inside the Nano-pores of Nano-porous materials. The potential of carrier materials, such as metal organic frameworks, meso-porous silica, controlled pore glass, porous polycyclohexyl ethylene and polystyrene, and Nanostructured lipid carriers, has been the subject of numerous investigations. (20) O'Mahony et al. created a method for filling the pores of CPG with API solution in order to produce Nano-crystalline products inside the controlled pore glass (GPG). To enable full filling of the CPG pores throughout the process, the inhibition time was calculated using the Washburn equation. The migration of this liquid-air interface with time t within a channel is typically described by the Washburn equation, which predicts the capillary flow of liquids in porous material.

$$L^2 = \frac{\gamma D t}{4 \mu \eta}$$

Where *t* is the time for a liquid of viscosity η and surface tension γ to penetrate a distance L into a fully wettable, porous material whose average pore diameter is D (21)

**B. Top down techniques** - The top down approach is the most important technique for the production of Nano-crystals. There are two fundamental top-down methods for reducing size: milling and high-pressure homogenization. The majority of Nano-crystal goods that have made it to market are made by wet milling. Wet milling is mechanical attrition in which milling balls in a milling container shear and grind particles that have been wetted by an aqueous solution of surfactants. Although the particle size is decreased and may even reach a few hundred micro-meters, Nano-sized crystals can be produced using modified conventional milling. One can perform the preparatory procedure in a repeatable way. The technique's main shortcomings are its high energy input, extended operating time, lower crystallinity, and contamination from metal milling ball or pearl erosion. (7) Polymeric beads could be useful in reducing contamination and erosion. The process of two fluid streams of particle suspensions colliding under high pressure in a chamber, causing particle collision and subsequent particle rupture, is known as high-pressure homogenization. In piston-gap homogenizers, a dispersion of drug particles is forced through a thin gap under high pressure using a piston, producing Nano-sized solid particles. The particles are fractured by strong shear forces and turbulent flow, and the number of piston-moving cycles, particle hardness, and homogenization power determine the particle outcome. The main disadvantages of high-pressure homogenization are that it necessitates a high process temperature, a large energy input, sophisticated equipment, and the potential for component degradation and lower yields compared to wet milling. The introduction of solid Nanoparticles to the market is hampered by the lack of a large-scale production process that produces a product of a quality that is acceptable to the regulatory authorities. Many sectors, notably the pharmaceutical sector, use high-pressure homogenizers and wet milling to produce micro and Nanoparticles. Therefore, wet milling and high-pressure homogenizers are seen to be the most practical industrial methods for producing Nano-crystals without any issues with regulations. (7)

B.1. Pearl/Ball milling - This method involves feeding the medication into the milling chamber together with the stabilizer, dispersion media (usually water), and milling media. Milling media might be miniature pearls or milling balls. Particle size is reduced as a result of the intense shear and impact forces produced by the milling medium movement. Merisko-Liversidge et al. created this technology (2003). (8) Ceramic (cerium or yttrium stabilized zirconium dioxide), glass, stainless steel, or beads covered in strongly cross-linked polystyrene resin make up the pearls or balls. The two basic principles of milling are employed. Either the milling material can be moved by an agitator or the complete container may be moved in a complex movement. In the latter method large batches are difficult to process, so mills using agitators are generally preferred for large batches. Milling time, however, depends upon various factors such as hardness of the drugs, surfactant contents, viscosity, temperature, energy input and size of the milling media. From thirty minutes to many hours, the milling process can take place. (8)The low cost, straightforward technique, and capacity for large-scale manufacturing are benefits of pearl milling. Long milling times (for hard drugs), the possibility of germ growth in the water phase (when milling for a long time), adherence of the product to the inner surface of the mill and to the surface of the milling pearls, erosion from the milling material that results in product contamination, and the time and expense involved in separating the milling material from the drug Nanoparticle suspension—particularly when producing parenteral sterile products—are the drawbacks of this process. (7, 8)

B.2. High Pressure Homogenization (HPH) - This method has been used for many years to create suspensions and emulsions. One unique benefit of this technology is how simple it is for increase in size.
Three key technologies for homogenization-based Nano-crystal production are as follows:

B.2.1. Micro fluidizer technology (IDD-PTM technology) - The jet-stream principle is the foundation of this technology. High pressures (up to 1700 bars) cause a frontal collision between two streams of liquid moving at high speeds (up to 1000 m/sec). High shear force particle collision and cavitation25 lower the particle size. Jet stream homogenizers like the micro-fluidizer (Micro-fluidizer Microfluidics Inc.) can do the same thing. The shape of the collision chamber might be either Z-type or Y-type. To maintain the appropriate particle size, phospholipids or surfactants are needed. Drug

Nano-suspensions for soft medicines can be made with a micro-fluidizer. However, because it takes a lot of cycles (50 to 100 passes) to achieve adequate particle size reduction, this method is not very practical for large-scale production. SkyePharma Canada Inc. uses this method, which they call IDD-P TM (Insoluble Drug Delivery Particle technology), to produce submicron particles of poorly soluble medications. (3)

B.2.2 .Piston gap homogenization in water (Dissocubes technology) - Müller et al. created piston gap homogenization technology, which SkyePharma purchased in 1999. This method involves dispersing a powdered medication in an aqueous surfactant solution, which is subsequently pushed through a microscopic homogenization gap under high pressure by a piston. The gap width is typically between 5 and 20 μm in size and is modified based on the suspension's viscosity and the applied pressure. The consequent high streaming velocity of the suspension raises the dynamic pressure, which is offset by a decrease in the static pressure, according to the Bernoulli equation. At room temperature, the static pressure in the gap is less than the water's vapour pressure. Thus, at room temperature, water begins to boil in the gap, causing gas bubbles to form. Pressure waves because the crystals to disintegrate as gas bubbles develop. Gas bubbles burst and the static pressure rises to normal air pressure when the liquid exits the homogenization gap. (3) Cavitation is the process by which gas bubbles develop and burst. High shear pressures, turbulent flow, and the tremendous power of these shock waves all contribute to the reduction of particle size. Using Tween 80 as a stabilizer, this method has been utilized to create artemisinin and quercetin Nano-suspensions (0.5–2.5% w/w). Some drawbacks of using water as a dispersion medium include issues with the drying process and the hydrolysis of medications that are sensitive to water. The removal of water from medications that are thermolabile or have a low melting point requires the employment of costly procedures like lyophilization. Therefore, dissocubes technology works best when creating aqueous suspensions of Nano-crystals for medications that are insoluble in both organic and aqueous media. The ability to produce Nano-suspensions for parenteral application aseptically is another benefit of this technique. This method has been used to create Nano-crystal solutions of cyclosporine, paclitaxel, amphidicolin, bupravaquone, azodyecarbonamide, and prednisolone. This method's two primary disadvantages are the expensive equipment installation and maintenance costs and the need for medication pretreatment (such as micronization).(3, 22)

B.2.3. Piston gap homogenization in water mixtures or in non-aqueous (Nano-pure technology) - The Nanopure technology, owned and developed by Pharmasol GmbH in Berlin, is an additional method that uses a piston-gap homogenizer. Non-aqueous phases or phases with a lower water content are used as dispersion medium in this technology. It is beneficial to use non-aqueous media for medications that hydrolyze in water. Oils, water-glycerol mixes, polyethylene glycols, water-alcohol mixtures, and other media are among the several ones utilized for homogenization. The vapor pressure of this dispersion media is low. The liquid does not boil and cavitation does not happen because the static pressure in the homogenization gap does not drop below the liquid's vapor pressure. Enough size reduction to the micro range occurs even in the absence of cavitation. Particle collision and shear forces in a very turbulent fluid in the gap are the forces causing size reduction. (3) Using Nano-pure technology for homogenization at lower temperatures—that is, below the freezing point of water—is comparable to or more effective. It is also possible to employ melted non-aqueous matrices, like PEG 6000, which are solid at room temperature, as a homogenization medium. This reduces crystal interaction and consequent crystal development while fixing drug Nano-crystals in the solid matrix. It is possible to directly fill gelatin or HPMC capsules with medication Nano-crystals suspended in liquid PEGs (such Miglyol 812 or 829) or oils. In order to create solid dosage forms like pills and pellets, Nano-crystals have been utilized as powder. Dispersion medium must be extracted from the Nano-crystals in order to generate solid oral dosage forms from the Nano-crystal solution. Spray drying or freeze drying are the methods used to remove the dispersion medium. Because Nanopure technology uses non-aqueous media or water-reduced combinations, evaporation occurs more quickly and at a lower temperature, which gives it an advantage in this situation. For medications that are thermolabile, this is helpful. (23)

B.3. Combination technology - Technologies that combine a pre-treatment phase with a high energy homogenization step have been referred to as combination technologies.

B.3.1. NANOEDGE Technology - (Homogenization and Micro-precipitation). When Baxter first created NANOEDGE Technology, it combined the precipitation and annealing processes. High energy, such as high shear forces and/or heat energy, is used in the annealing process. (24) The precipitated Nanoparticles have a propensity to proliferate when drug Nanoparticles are made only by the precipitation approach. Additionally, the precipitated particles could be partially or completely amorphous. The amorphous particles may re-crystallize during storage, which could reduce the drug's bioavailability. On the other hand, because the annealing process turns all precipitated particles into crystalline states, combination technology has the potential to solve these issues in two ways: first, by preventing crystal growth, and second, by lowering the uncertainty of forming either a crystalline or amorphous state. Drugs like N-methyl-2-pyrrolidinone that dissolve in non-aqueous fluids and have low toxicity are very well suited for Nanoedge technology. However, the cost of this approach is a disadvantage, particularly when preparing sterile parenteral products. (3)

B.3.2. SmartCrystal Technology - It is a toolbox of several combination procedures whereby process variants can be selected based on the drug's physical properties (e.g., hardness). HPH is combined with spray-drying in the H42 process. One to a few homogenization rounds can create drug Nano-crystals considerably more quickly. Amphotericin B Nano-crystals with a size range of roughly 50 nm are produced by processes H69 (precipitation and HPH) and H96 (lyophilization and HPH). Ball-milling and combination processes were used to create cosmetic active hesperidin Nano-suspensions. Both of the Nano-suspensions that were made were stored. It was discovered that the Nano-suspension made with SmartCrystal technology was smaller, suggesting greater physical stability. Additionally, compared to HPH alone, the combination approach is quicker and more cost-effective. (3, 24)

## Types of Nano-cryastals:

### 1. Organic Nano-cryastal:

Organic NCs are microscopic structures composed of organic compounds like hemoglobin, cellulose, and 35 medications (such as paclitaxel, resveratrol, and cabazitaxel) and polymers. Mechanical milling is one of the often employed techniques for the synthesis of organic NCs. These are commonly created by condensation and precipitation procedures that involve bottom-up techniques or synthetic methods or self-assembly principles. Additional synthesis techniques include sol-gel coatings, sonication, solvent-vapor annealing, laser ablation, and sol-gel. Organic NCs are typically larger than 100 nm and up to 1 mm in diameter. The size and shape of the produced NCs are modulated in large part by the organic molecules. To produce homogenous cellulose NCs, a number of techniques are commonly employed, such as oxidation, which breaks the cellulose microfiber and disrupts the hydrogen bonding network, acid hydrolysis, and enzyme-assisted hydrolysis. (25, 26) Using the amide linkage employed for anticancer medication treatment, cellulose NCs were created in one study by joining a carboxylic group with presynthesized cis-aconite-doxorubicin. By increasing their bioavailabilities and lowering their toxicities, organic medications and compounds such 10-hydroxycamptothecin, oridinon, ursolic acid (UA),paclitaxel, and silybin48 have been used to treat cancer in the form of NCs. Zhou et al. created hemoglobin NCs using the batch crystallization technique in 2023 and applied them to colon tumor-specific diagnosis and treatment. All things considered, hemoglobin NCs (HbC) and other biological and organic NCs have benefits such low toxicity, cell targeting, superior tissue penetration, biodegradability, and ease of manipulation. However, the main disadvantages of organic NCs are their size, tissue buildup, allergic reactions, and effectiveness. (27)

### 2. Inorganic Nano-cryastal:

Organic substances include metals, ceramics, magnetic materials, semiconductors/metalloids, and metal oxides. These are between 1 and 100 nm in size. These NCs have drawn a lot of interest for therapeutic and diagnostic applications due to their unique optical, magnetic, and fluorescent properties. Their characteristics and nature have allowed them to be distinguished from one another. Made of semiconductor materials,

quantum dots have size-dependent optical characteristics including adjustable wavelengths for absorption and emission. They are therefore useful for biological imaging, solar cells, displays, illumination, and sensing applications. (28) V. Bajpai et al. produced nitrogen phosphorus-doped quantum carbon dots with anticancer characteristics using PEG, imidazole, and phosphoric acid. These carbon quantum dots cause B16F10 melanoma cancer cells to die by exhibiting apoptotic characteristics such as cell cycle arrest and autophagy. 30 A variety of metals, including copper, silver, platinum, and gold, can be used to create metal NCs. They have increased light absorption and dispersion because to their localized surface plasmon resonance (LSPR). They can be used in biological sensing, electronics, photonics, and catalysis. A. McGrath et al. used the seed growth approach to manufacture palladium NCs coated with gold. By eliminating HeLa cells in vitro and HeLa tumor tissues in vivo, they demonstrate effective photothermal applications. Cobalt (Co), gadolinium, or iron oxides make up magnetic NCs. Depending on their size, they behave either ferromagnetically or superparamagnetically. They are employed in medication delivery, magnetic resonance imaging (MRI), data storage, and magnetic hyperthermia in cancer theranostics. Superparamagnetic iron oxide (Fe2O3 and Fe3O4) is one such instance. Iron oxide (IO) Nano-crystals are used in a variety of biomedical applications, including protein extraction, therapeutic gene carriers, MRI contrast agents, and the detection of viruses and nucleic acids. IONCs with a hyaluronic acid coating were created using the thermal decomposition approach for targeted cancer imaging, according to a paper by Y. Lee et al. In a different work, IO NCs were synthesized using the coprecipitation and partial oxidation techniques for use in MR and NIR absorption imaging. It is simple to modify inorganic NCs to any size or shape. Inorganic NCs have greater surface area and drug loading capacity since they are smaller than organic NCs. Furthermore, it is not suited for cancer theranostics due to its lack of biocompatibility and increased cytotoxicity in both animal models (in vivo) and cells (in vitro). (27)

**3. Hybrid Nano-crystals:**

The remarkable imaging ability of hybrid NCs, in which drug crystals act as hosts and combine with molecules of interest to form composites, has drawn a lot of attention. The idea is based on a well-known phenomenon in solid-state chemistry whereby impurities change the optical, mechanical, and electrical characteristics of a host by integrating into its crystal structure. (29) Nevertheless, the crystallinity and biological functional characteristics of drug Nano-crystals are not significantly impacted by incorporated dye molecules. Scientists have created a number of hybrid NCs over the course of ten years utilizing a variety of medications, including cyclosporine, lapatinib, camptothecin, paclitaxel, quercetin, itraconazole, and saquinavir. For the purpose of cancer imaging, dopants such as rhodamine, Cy5, gold, DiD, DiR, FPI-749, FPR-749, FPR-648, and fluorescein were added to the host particles. By incorporating Au into the host crystal, C. P. Hollis et al. reported synthesizing organic camptothecin-doped Au NCs. In this case, gold serves as an image contrast agent for bioimaging while camptothecin aids in the death of colon cancer cells. (6)Another work created luminous organic–inorganic hybrid NCs by preparing hydroxyapatite NCs with tris(2,2,6,6-tetramethyle-3,5-heptanedionato) europium(III) (EuTH) complex. Additionally, these NCs were coupled with variants of folic acid to target HeLa cancer cells. (27)

## Application of Nano-crystals to Pharmaceuticals in Drug Development:

1. Parent management/ administration
2. By oral administration
3. Delivery of the drug via the lungs
4. Targeted drug delivery
5. Skin administration of the drug

1.) Parent management/ administration: Numerous parenteral routes of administration, including intra-articular, can be used to deliver drug Nano-crystals in the form of Nanosuspensions. Injection into the abdominal cavity into the veins. It has been demonstrated that Nanosuspension increases the efficacy of parenteral medications. Compared to liposomal clofazimine, clofazimine

Nanosuspension, an anti-leprosy medication that is less water soluble, is more stable and efficient. (31, 32, 33)

2.) By Oral administration: The drug's oral absorption and subsequent bioavailability are significantly enhanced by Nanosizing it. Tablets and hard gelatin capsules with pellets are two examples of liquid dosage forms that can directly use aqueous Nanosuspension. (31, 32, 33)

3.) Pulmonary drug delivery: Both mechanical and ultrasonic nebulizers can be used to nebulize aqueous Nano-crystals for lung administration. Because there are numerous small particles rather than a few microparticles, the dispersion can have a high concentration; all aerosol droplets contain medication Nano-crystals. Budesonide, a corticosteroid that is poorly soluble in water, has been effectively made into a Nanosuspension for use in nebulization to treat lung infections. (31, 32, 33)

4.) Target drug delivery: Target delivery can be accomplished with Nano-crystals. Using a surface-modified mucosal adherent Nanosuspension of buplavauon, the causative organism of cryptosporidium illness, Cryptosporidium parvum, was targeted. In a similar vein, illnesses like pulmonary aspergillosis can be readily treated by substituting lung Nanosuspensions of suitable medication candidates, like amphotericin B, for stealth liposomes. (31, 32, 33)

5.) Skin drug delivery: If the traditional formulation method is unsuccessful and the application of active ingredient Nano-crystals results in a greater concentration gradient between the formulation and the skin, skin Nanosuspensions are particularly intriguing. Supersaturated formulations produced by increased saturation solubility improve medication absorption through the skin. By stabilizing drug Nano-crystals with a positively charged polymer, this impact can be further increased. Drug Nano-crystals have a greater affinity for negatively charged stratum conium when their charges are opposite. (31, 32, 33)

## Application:

When it comes to the distribution of pharmaceutical medications in various contexts, the NC approach has a lot to offer. Therefore, following a description of NCs' extensive adaptability to different administration routes and biological applications, examples of already-marketed NC products are provided

- The generation of hydrogen
- Removal of poisons and pollutants
- Diagnostic imaging
- Biotags for identifying genes
- Drug production • Protein evaluation
- Flat-panel televisions
- Illumination
- Infrared and optical lasers
- Optoisolators
- Chips for magneto-optical memory
- Self-contained intelligent materials.

•Nanoscale surface treatments or additives can help materials in personal body armor provide lightweight ballistic energy deflection and resist bacterial growth, stains, and wrinkles. (34)

•Washable, durable "smart textiles" with pliable Nanoscale sensors and electronics that can track health, capture solar energy, and collect energy through movement are becoming feasible thanks to Nanoscale materials. (35)

**Nano-crystals characterization:**

1) Particle Size Analysis: A particle size analyzer called Nanotrac 150 (Japan) with a wet sampling system dynamically scatters light after the size and size distribution of dried morphological crystals are redispersed in water that contains 0.1% polyvinyl alcohol (PVA and 403). Determined by using the reported average particle size distribution to derive the diameter. (33)
2) Determining drug content: To verify the produced sample's purity, the drug content of the lyophilized sample was examined using a UV and spectrophotometer. The product's aqueous dispersion (25 mg/10 ml distilled water) was run through a 0.8 µm filter in order to measure the amount of medication present. Using spectrophotometry at a wavelength of 291 nm, the drug concentration was determined after a filtrate containing fine particles less than 0.8 µm was dissolved in a 4% sodium lauryl sulfate solution. The Nano-crystal yield, which represents the ratio of drug infiltration to total drug in the dispersion, was computed. (33)
3) Scanning electron microscopy: SEM was used to analyze the surface morphology of the freeze-dried formulation samples and the commercial medication powder. The samples were coated with 80 nm gold/palladium in a Blazers 120B sputtering apparatus and placed on metal discs covered with double-sided adhesive carbon tape prior to inspections. (33)
4) Powder X-ray diffraction (PXRD): An X-ray diffractometer (PW 1729, Philips, Netherlands) was used to record the XRD patterns. Monochromatized Cu-Ka radiation (1.542A˚) was used to irradiate the samples, and measurements were made between 50 and 500 2θ. 30 kV and 30 mA of voltage and current were utilized, respectively. Total scattering and scattering from the crystalline region of formulations and pure pharmaceuticals were measured as the foundation for the XRD process, which was used to assess the degree of crystallinity. (33)
5) Differential scanning calorimeter: The thermal behavior of the commercial griseofulvin powder and the freeze-dried samples was measured using a DSC equipment that was fitted with a liquid nitrogen cooling system. Aluminum pans containing 2 and 5 mg samples were subjected to DSC analysis at a scan rate of 100 °C per minute between 25 and 300 °C. (33)
6) Solubility: Using a UV spectrophotometer, UV absorbance data at 291 nm were used to analyze saturation solubility results. 150 ml of 4% SLS solution was mixed with excess griseofulvin powder and the formulation, and the combination was shaken in a mechanical shaker for 24 hours at 37 ± 0.05. degrees C, using a shaker GLF1086. To ensure that the surplus sample was present in the solid state and had reached saturation, a thorough visual inspection was conducted. A 0.2 µm filter was used to filter the combination, the filtrate was suitably diluted, and the amount of griseofulvin soluble in each formulation was quantified. (36)
7) Dissolution test: Reinhard gelatin capsules were used to evaluate the dissolution of griseofulvin and the commercially available medication (Zydus Cadila, Goa, India). The medication powder and prepared sample were placed in 125 mg capsules, heated to 37 ± 0.5 °C, and then put through a dissolving test with 900 ml of 4% SLS solution as the dissolution medium. A speed of 75 rpm was used to rotate the basket. At certain intervals, samples of at least 10 ml were collected, filtered through a 0.2, and their concentration was determined using spectrophotometers and UV light. (36)
8) Stability study: The formulations are separated into two sections: 30 ° C ± 2 ° C and 65% ± 5% relative humidity, and 40 ° C ± 2 ° C and 70% ±. All formulations are assessed for stability in accordance with ICH criteria. It was kept there. Keep the relative humidity at 5%. After a predetermined amount of time, estimates of drug release and content were made. (36)

## History of product (Nano-crystals) on the market and Clinical Phases:

Only a small number of Nanoparticle delivery techniques reach the market, while many are being studied in academia. The duration between technological development and the first products available on the market is significantly shorter than for other submicron drug delivery technologies. The business Wyeth released Rapa-mune, its first medicine, on the market in 2000, just ten years after the drug Nano-crystals concept was first introduced. These tablet drug Nano-crystals perform better than the previous formulation (oral solution), as evidenced by their significantly (21%) increased bioavailability. Emend was created as an NCE (new chemical entity) in a Nano-crystal formulation and introduced by Merckin in the United States in April 2003. Abott introduced TRICOR 145mg and 48mg in the United States in 2004. The bioavailability of the new formulation was able to increase by 9%. Megace ES (2005) uses Nano-crystal technology to increase the oral megestrol acetate suspension's bioavailability and rate of dissolution. Using Nano-crystal technology as well, Invega Sustenna (paliperidone palmitate, Janssen) has created one commercial product: Triglide (Skye Pharma, fenofibrate) in 2009. In addition to these medicines, almost 20 more that use drug Nano-crystal technology are still in the trial phase. (37)

Drug Nano-crystal technology still has many drawbacks despite its many advantages. First, the lack of information on Nanotoxicity was caused by the tiny size of Nano-crystals, which allowed them to infiltrate any cell in the body through pinocytosis and cause additional cytotoxicity. Second, the cost of the final medicine will increase due to the necessity for costly equipment for this new procedure. Furthermore, because this strategy is only used on BCS class II, it cannot yet be considered a completely ubiquitous approach. Lastly, the molecular drug structure determines the stability and manufacturing of various drug Nano-crystals. (37)

## Conclusion:

Drug Nano-crystals are an important formulation approach for poorly water-soluble medicines. They can be used in many routes of administration for API delivery. The Nano-crystal technology provides great improved surface area provides benefits such as improved saturation solubility and dissolving velocity. First, we discussed Nano-crystal preparation methods and recent advances in them. Milling and high-pressure homogenization are effective processes for producing the majority of Nano-crystal items on the market. Further the types of Nano-crystals were explored along with its characterisation. Applications and marketed products of Nano-crystals are described. In the future, Nano-crystal surfaces can be adjusted for prolonged or targeted release of Nanoparticles, they can be modified as per the need of drug delivery systems which is crucial for their effectiveness.

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