A NANOCRYSTAL TECHNOLOGY: TO ENHANCE SOLUBILITY OF POORLY WATER SOLUBLE DRUGS

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ABSTRACT
Most of the recently developed new chemical entities are poorly water soluble and they create major problems during formulation and development of new dosage form and due to poor solubility and poor bioavailability. The drugs belong to BCS class II and class IV has problem of solubility, to overcome the solubility problem nanotechnology is most useful technique. In this review article the main focus on Nanocrystals and various techniques used for preparation of Nanocrystals. Drug nanocrystals consists pure poorly water soluble drugs without any matrix material which means that it is carrier free drug delivery. Nanocrystals technologies have been introduced as advantageous, universal formulation approaches for the BCS class II and IV drugs. Nanocrystals, with greater surface to volume ratio, can effectively increase both the dissolution rate and saturation solubility of active ingredients The Nanocrystals is suitable drug delivery system for all commonly used routes of administration such as oral, IV, SC, and IM and topical application. Nanocrystals can also be incorporated into the tablets, capsules, fast-melts and lyophilized for sterile product applications. There are no of techniques which are used for production including precipitation, milling, high pressure homogenization and combination methods such as Nano-Edge, SmartCrystal and Precipitation-lyophilization-homogenization (PLH) technology.

INTRODUCTION
Currently one of the main applications of nanotechnology in drug delivery is to overcome the problem of poor water solubility of hydrophobic drugs. Approximately, 40% of all developmental new chemical entities are poorly water soluble and therefore, are difficult to formulate [1]. The poor solubility of drug limits the development of highly potent drug formulation. The drugs with low solubility lead to low oral bioavailability and erratic absorption which is particularly pertinent to drugs within class II of the Biopharmaceutical Classification System (BCS). Generally, the rate-limiting step for absorption of the drugs in this class is the dissolution velocity arising from low solubility. Although the drugs are high permeability, the poor solubility results in a low concentration gradient between gut and blood vessel consequent to a limitation of drug transport and oral absorption. Nowadays, there are a large percentage of drug compounds in drug development represents as poor aqueous solubility. Conventional formulations of poorly-water soluble drugs are frequently facing the problems such as poor and highly variable bioavailability. The dosage form is often times affected by the fed–fasted state of the patient and its onset of action is slower than anticipated. All of these issues lead to sub-optimal dosing and poor performance. Therefore, one of the most challenging tasks in drug development is to improve the drug solubility in order to enhance the bioavailability of these drugs. The approaches to increase the solubility and the available surface area for dissolution are classified as physical and chemical modifications. For the physical modification, the techniques include decreasing particle size (micronization, nanonization), formation of polymorphs/pseudo polymorphs (including solvates), complexation/ solubilization (by means of using...
surfactants or cyclodextrins, conjugation to dendrimers, and an addition of co-solvents) and preparation of drug dispersions in carriers (eutectic mixtures, non-molecular solid dispersions, solid solutions). For the chemical modification, the used technique is the synthesis of soluble prodrugs and salts. The micronization of drugs is applied to increase their surface area. Increasing the surface area will proportionally increase the rate of dissolution and the rate of diffusion (absorption). Micronization cannot improve the saturation solubility of a drug substance. Consequently, the next step was taken to move from micronisation to nanonisation that means producing drug nanocrystals. [2] Drug nanocrystals are crystals with a size in the nanometer range, which means they are nanoparticles with a crystalline character. There are discussions about the definition of a nanoparticle, which means the size of a particle to be classified as a nanoparticle, depending on the discipline e.g., in colloid chemistry particles are only considered as nanoparticles when they are in size below 100 nm or even below 20 nm. Based on the size unit, in the pharmaceutical area nanoparticles should be defined as having a size between a few nanometers and 1000 nm (=1 μm); micro particles therefore possess a size of 1–1000 μm [3, 4]. A further characteristic is that drug nanocrystals are composed of 100% drug; there is no carrier material as in polymeric nanoparticles. Dispersion of drug nanocrystals in liquid media leads to so called “Nanosuspensions” (in contrast to “micro suspensions” or “macro suspensions”). In general the dispersed particles need to be stabilized, such as by surfactants or polymeric stabilizers. Dispersion media can be water, aqueous solutions or nonaqueous media (eg, liquid polyethylene glycol [PEG], oils). Depending on the production technology, processing of drug microcrystals to drug nanoparticles can lead to an either crystalline or to an amorphous product, especially when applying precipitation. In the strictest sense, such an amorphous drug nanoparticle should not be called nanocrystals. However, often one refers to “nanocrystals in the amorphous state.” [5, 6]

**ADVANTAGES OF NANOCRYSTALS**

- It can be given by any route of administration.
- Enhanced solubility and bioavailability of drug.
- Reduced tissue irritation in case of subcutaneous/intramuscular administration.
- Rapid dissolution & tissue targeting can be achieved by IV route of administration.
- Oral administration of Nano suspension provide rapid onset, reduced fed/fasted ratio & improved bioavailability.
- The absorption form absorption window can be increased, due to reduction in the particle size.
- It can be incorporated in tablets, pellets, hydrogel & suppositories are suitable for various routes of administration.
- Increasing the amorphous fraction in the particles leading to a potential change in the crystalline structure & higher solubility.
- Possibility of surface-modification of Nanosuspension for site specific delivery.
- Higher drug loading can be achieved.
- Long term physical and chemical stability (due to absence of Ostwald ripening)[7,8]

**DISADVANTAGES OF NANOCRYSTALS**

- Physical stability, sedimentation & compaction can cause problems.
- It is bulky sufficient care must be taken during handling & transport.
- Uniform & accurate dose cannot be achieved[7,8]

**PROPERTIES OF NANOCRYSTALS**

- Increase of dissolution velocity by surface area enlargement

The size reduction leads to an increased surface area and thus according to the Noyes-Whitney equation to an increased dissolution velocity. Therefore micronization is a suitable way to successfully enhance the bioavailability of drugs where the dissolution velocity is the rate limiting step. By moving from micronization further down to nanonization, the particle surface is further increased and thus the dissolution velocity increases too. In most cases, a low dissolution velocity is correlated with low saturation solubility. Noyes and Whitney Equation:

\[ \frac{dc}{dt} = \frac{DA}{h}(Cs - Cx) \]

Where, \( \frac{dc}{dt} \) is dissolution velocity, \( D \) is diffusion coefficient, \( A \) is surface of the drug particle, \( h \) is thickness of diffusional layer, \( C_s \) is saturation solubility of the drug and \( C_x \) is concentration in surrounding liquid at time

- Increase in saturation solubility

The saturation solubility \( C_s \) is a constant depending on the compound, the dissolution medium and the temperature. This is
valid for powders of daily life with a size in the micrometer range or above. However, below a critical size of 1–2 μm, the saturation solubility is also a function of the particle size. It increases with decreasing particle size below 1000 nm. Therefore, drug Nanocrystals possess increased saturation solubility. This has two advantages:

i. According to Noyes and Whitney equation, the dissolution velocity is further enhanced because dc/dt is proportional to the concentration gradient (Cs−Cx)/h (Cs- saturation solubility, Ct - bulk concentration, h- diffusional distance).

ii. Due to the increased saturation solubility the concentration gradient between gut lumen and blood is increased, consequently the absorption by passive diffusion.

The saturation solubility of solid particles depends on their particle radius and their lattice structure according to the Ostwald-Freunlich equation and the Kelvin equation

$$\ln\left(\frac{S}{S_0}\right) = \frac{2\nu}{rRT} - \frac{2M\gamma}{\nu rRT}$$

Where, S is the drug solubility at temperature T, S₀ the solubility if r = ∞, M the molecular weight of the compound, ν the molar volume, γ the interfacial surface tension, and ρ the density of the compound. From the Ostwald-Freunlich equation, it can be concluded that a drug shows higher solubility if the particle radius is decreased. This effect is not substantial for larger particles but will be more pronounced for particles below 1 to 2 μm, especially well under 200 nm. Another important factor influencing the solubility is the crystalline structure of the drug. The higher the solid density and the melting point are, the lower the solubility. In contrast, a polymorph form with a lower packaging shows a higher molar volume and lower solid density.⁹,¹⁰ The Kelvin equation can also be used to describe the correlation of increased saturation solubility by decreased particle size. The Kelvin equation describes the vapor pressure as a function of the curvature of liquid droplets in a gas phase. The vapor pressure increases with increasing curvature (decreasing particle size).⁹

Techniques for Manufacturing of Nanocrystals

1. **Bottom up technology**
   1.1 Anti-solvent precipitation
   1.2 Supercritical fluids
   1.3 Spray-drying

2. **Top down Technology**
   2.1 Media milling
      2.1.1. Bead milling
   2.1.2. Dry co-grind
   2.2 High pressure homogenizations
      2.2.1. Homogenization in Aqueous media (Disso cubes)
   2.2.2. Homogenization in Non Aqueous Media (Nanopure)
   2.2.3 Nanojet technology
   2.3 Emulsion solvent diffusion method

3. **Combination technology**
   3.1 NANOEDGE® Technology
   3.2 SmartCrystal® Technology

4. **Other methods**
   4.1. Solvent evaporation
   4.2. Sonocrystallization
   4.3. Melt emulsification
   4.4. Bottom-Up NanoCrySP Technology

1. **Bottom up technology**
Principal of this technology is based on precipitation by dissolving the drug in a solvent and adding the solvent to a non-solvent that cause precipitation of the fine drug particle.

1.1 **Precipitation technology (Antisolvent method):**
In this technique the drug is dissolved in an organic solvent in which it is soluble and this solution is mixed with a miscible Antisolvent for precipitation in presence of stabilizer. In the water-solvent mixture the solubility is low and the drug precipitate out. Precipitation has also been combined with high shear processing. This is accomplished by a combination of rapid precipitation and high-pressure homogenization. The Baxter healthcare company introduced their patented technology US 6,884,436 known as NANOEDGE. This technology based on precipitation of friable materials for fragmentation under conditions of high shear and/or thermal energy. Sudden super saturation of the mixed solution occurs by rapid addition of drug solution to Antisolvent and generation of fine crystalline or amorphous solids. Precipitation of an amorphous material may be favored at high super saturation when the solubility of the amorphous state is exceeded. Precipitation method is the preparation of amorphous drug nanoparticles, for example, as carotene nanoparticles in the food industry, e.g., Lucarotin® or Lucantin® (BASF). A solution of the carotenoid, together with a surfactant in a digestible oil, are mixed with an appropriate solvent at a specific temperature. To obtain the solution a protective colloid
is added. This leads to an O/W two phase system. The carotenoid stabilized by the colloid localizes in the oily phase. After lyophilization X-ray analyzes shows that approximately 90% of the carotenoid is in an amorphous state.\textsuperscript{[10]}

1.2 Supercritical fluid methods
Nanoparticles are produced by various methods like rapid expansion of supercritical solution (RESS) process, supercritical Antisolvent process, and precipitation with compressed Antisolvent (PCA) process. In RESS technique, drug solution is expanded through a nozzle into supercritical fluid, resulting in precipitation of the drug as fine particles by loss of solvent power of the supercritical fluid. Young et al. prepared cyclosporine nanoparticles having diameter of 400 to 700 nm by using this technique. In the PCA method, the drug solution is atomized into the CO\textsubscript{2} compressed chamber. As the removal of solvent occurs, the solution gets supersaturated and finally precipitation occurs. In supercritical Antisolvent process, drug solution is injected into the supercritical fluid and the solvent gets extracted as well as the drug solution becomes supersaturated. The basic disadvantages of this methods are use of hazardous solvents and use of high proportions of surfactants and stabilizers as compared with other techniques.

1.3 Spray drying
This method is usually used for drying of solutions and suspensions. In a conical or cylindrical cyclone, solution droplets are sprayed from top to bottom, dried in the same direction by hot air and spherical particles are obtained. Spraying is made with an atomizer which rapidly rotates and provides scattering of the solution due to centrifugal effect. The solution, at a certain flow rate, is sent to the inner tube with a peristaltic pump, nitrogen or air at a constant pressure is sent to the outer tube. Spraying is provided by a nozzle. Droplets of solution become very small due to spraying; therefore, surface area of the drying matter increases leading to fast drying. Concentration, viscosity, temperature and spray rate of the solution can be adjusted and particle size, fluidity and drying speed can be optimized. The dissolution rate and bioavailability of several drugs, including hydrocortisone, COX-2 Inhibitor (BMS-347070) were improved utilizing this method.\textsuperscript{[11]}

2. Top down technology
The ‘Top down Technologies’ are the disintegration methods and are preferred over the precipitation methods.

2.1 Media milling (Nanocrystals or Nano systems)
2.1.1. Bead milling: The method is first developed by liversidge et al. In this method the Nanosuspensions are produced using high-shear media mills or pearl mills. The media mill consists of a milling chamber, a milling shaft and a recirculation chamber. Shear forces of impact, generated by the movement of the milling media, lead to particle size reduction of several drugs, including hydrocortisone, COX-2 Inhibitor (BMS-347070) were improved utilizing this method.\textsuperscript{[11]}

2.1.2. Co-grinding
Stable Nanoosuspensions are formulated using dry grinding of poorly soluble drugs with soluble polymers and copolymers after dispersing in a liquid media. It is the colloidal particles formation of many poorly water soluble drugs; griseofulvin, glibenclamide and nifedipine obtained by grinding with polyvinylpyrrolidone (PVP) and sodiumdodecylsulfate (SDS). Various soluble polymers and co-polymers such as PVP, polyethylene glycol (PEG), hydroxypropyl methylcellulose (HPMC) and cyclodextrins derivatives have been used. By using this method the physicochemical properties and dissolution of poorly water soluble drugs were improved because of an improvement in the surface polarity and transformation from a crystalline to an amorphous drug. Dry co grinding can be carried out easily and economically and can be conducted without need of organic solvents.
2.2 High Pressure Homogenization

When producing nanocrystals using homogenization methods, there are three important technologies namely: Microfluidizer technology (Nanojet technology), Piston gap homogenization in aqueous media (Dissocubes® technology) and in water mixtures or in nonaqueous media (Nanopure® technology)\(^2\).

2.2.1. Microfluidizer Technology (Nanojet technology)

This technology called opposite stream or Nanojet technology. This method consist of Microfluidizer which uses a chamber where a stream of suspension is divided into two or more parts, which colloid with each other at high pressure. This leads to particle collision, shear forces and also cavitation forces.

The high shear force produced during the process due particle collision and high pressure results in particle size reduction. Equipment using this principle includes the M110L and M110S micro fluidizers. Dearm prepared Nanoosuspensions of atovaquone using the micro fluidization process. The major disadvantage of this technique is the high number of passes through the micro fluidizer and that the product obtained contains a relatively larger fraction of microparticles.

2.2.2. Piston gap homogenization in aqueous media (Dissocubes)

This technology was developed by R.H.Muller in 1999 and first patent was taken by DDS Gmbh and afterward the patent was transferred to Skype pharmaceuticals. Commonly used homogenizer are the APVMicron Lab 40 (APV Deutschland Gmbh, Lubeck, Germany) and piston-gap homogenizers. In this method, the suspension containing a drug and surfactant is forced under pressure through a Nanosized aperture valve of a high pressure homogenizer. In this method the particle size reduction depend on cavitation principle.

The dispersion present in 3cm diameter cylinder is suddenly passed through a very narrow gap of 25µm. According to Bernoulli’s law the flow volume of liquid in a closed system per cross section is constant. It leads to increase in dynamic pressure and decrease of static pressure below the boiling point of water at diameter from 3cm to 25µm. Then water starts boiling at room temperature and forms gas bubbles, which implode when the suspension leaves the gap (called cavitation) and normal air pressure is reached. The particles cavitation forces are sufficiently high to convert the drug micro particles into nanoparticles. In this the final particle size of drug nanocrystals is based on power density of homogenizer, number of homogenization cycles, temperature and homogenization pressure.

2.2.3 Homogenization in Non Aqueous Media (Nanopure)

In this technology suspension is homogenized in water-free media or water mixtures. In the Dissocubes technology the cavitation is the principle determining factor of the process oils and oily fatty acids have very low vapour pressure and a high boiling point as compare to water. Hence, the drop of static pressure will not be sufficient enough to initiate cavitation. Patents covering disintegration of polymeric material by high pressure homogenization mention that higher temperatures of about 80°C promoted disintegration, which cannot be used for thermo labile compounds. In Nanopure technology, the drug suspensions in the non aqueous media were homogenized at 0°C or even below the freezing point and hence are called "deep-freeze" homogenization. The results obtained were comparable to Dissocubes and hence can be used effectively for thermo labile substances at milder conditions.\(^2\)

2.3. Emulsion solvent diffusion method

The use of emulsions as templates is applicable for those drugs that are soluble in either volatile organic solvent or partially water-miscible solvent. Such solvents can be used as the dispersed phase of the emulsion. An organic solvent or mixture of solvents loaded with the drug is dispersed in the aqueous phase containing suitable surfactants with stirring to form an emulsion. The obtained emulsion was further homogenized by high pressure homogenization. After homogenization cycles the emulsion was diluted with water, homogenized by homogenizer to diffuse the organic solvent and convert the droplets into solid particles.

Since one particle is formed in each emulsion droplet, it is possible to control the particle size of the Nanosuspension by controlling the size of the emulsion. Optimizing the surfactant composition increases the intake of organic phase and ultimately the drug loading in the emulsion. Originally methanol, ethanol, ethyl acetate, chloroform are used as organic solvents. However, environmental hazards and human safety concerns about residual solvents have limited their use in routine manufacturing processes. Nanosuspension of ibuprofen, diclofenac, and acyclovir were prepared by this method.
3. Patented Technologies

3.1 NANOEDGE™

The basic principles of NANOEDGE are the same as that of precipitation and homogenization. A combination of these techniques results in smaller particle size and better stability in a shorter time. The major drawback of the precipitation technique, such as crystal growth and long term stability, can be resolved using the NANOEDGE technology. In this technique, the precipitated suspension is further homogenized; leading to reduction in particle size and avoiding crystal growth. Precipitation is performed in water using water-miscible solvents such as methanol, ethanol and isopropanol. It is desirable to remove those solvents completely, although they can be tolerated to a certain extent in the formulation. For an effective production of Nanosuspensions using the NANOEDGE technology, an evaporation step can be included to provide a solvent-free modified starting material followed by high-pressure homogenization.[13]

3.2 SmartCrystal® technology

This technology was first developed by PharmaSol GmbH and was later acquired by Abbott. It is a tool-box of different combination processes in which process variations can be chosen depending upon the physical characteristics of the drug (such as hardness). The process H42 involves a combination of spray-drying and HPH. Within few homogenization cycles the nanocrystals is prepared. Process H69 (Precipitation and HPH) and H96 (lyophilization and HPH) yield nanocrystals of amphotericin B within a size range of about 50 nm. S. Kobierski et al. (2008) produced nanocrystals in a two-step process i.e. pre-milling followed by high pressure homogenization (HPH). Nanosuspensions of cosmetic active hesperidin were produced by ball milling process and with combination process. Prepared nanosuspensions were kept for storage. Nanosuspension prepared using SmartCrystal® technology was found to be of a smaller size indicating better physical stability.[13]

4. Other technologies

4.1 Solvent Evaporation

In this method, the solutions of polymer are prepared in volatile solvents and emulsions. But from last few years dichloromethane and chloroform were used which was now replaced by ethyl acetate which has a better profile of toxicology. The emulsion is converted into a nanoparticle suspension on evaporation of the solvent for the polymer, which is allowed to diffuse through the continuous phase of the emulsion. In the conventional methods, two main strategies are being used for the formation of emulsions, the preparation of single emulsions, e.g., oil-in-water (o/w) or double-emulsions, e.g., (water-in-oil)-in-water, (w/o)/w. These methods require high-speed homogenization or ultrasonication, followed by evaporation of the solvent, either by continuous magnetic stirring at room temperature or under reduced pressure. By ultracentrifugation the solidified nanoparticles are collected which was washed with distilled water to remove the additives like surfactants, and then it was lyophilized. The particle size was influenced by the concentration of polymer, stabilizer and the speed of homogenizer.

4.2 Sonocrystallization

The novel approach for particle size reduction on the basis of crystallization by using ultrasound is Sonocrystallization. Sonocrystallization utilizes ultrasound power characterized by a frequency range of 20-100 kHz for inducing crystallization. It not only enhances the nucleation rate but also an effective means of size reduction & controlling size distribution of the active pharmaceutical ingredient (API). Most applications used ultrasound in the range 20 kHz -5 MHz Sonocrystallization technique or technology has also been studied to modify the undesirables of NSAID’S i.e. poor solubility and dissolution rate and consequently the poor bioavailability.

4.3. Melt emulsification method

Solid lipid nanoparticles are mainly prepared by melt emulsification method. Kipp and coworkers firstly prepare Nanosuspensions of ibuprofen by using melt emulsification method. It is a four-step procedure. Drug is first added to aqueous solution having stabilizer. The solution is heated at temperature higher than the melting point of the drug and then homogenized by high-speed homogenizer for the formation of emulsion. The temperature is maintained above the melting point of the drug during overall process. Finally, the emulsion is cooled to precipitate the particles. The particle size of Nanosuspension mainly depends on parameters like drug concentration, concentration and type of stabilizers used, cooling temperature, and homogenization process.

4.4. Bottom-Up NanoCrySP Technology

G. Shete, Y. Pawar et al, National Institute of Pharmaceutical Education and Research (NIPER) introduced a newer method
to generate Nano crystalline solid dispersion (NSD) of hesperetin using NanoCrySP technology: a novel bottom-up process based on spray drying to generate solid particles containing drug nanocrystals dispersed in the matrix of small molecule excipients (WO2013132457 A2). The purpose of their study to improved oral bioavailability and pharmacodynamics activity of hesperetin nanocrystals generated using a novel bottom-up NanoCrySP Technology. Hesperetin and mannitol were used in 1:1 ratio and NSD was generated using spray drying. The process of NSD formation is based on classical nucleation theory wherein mannitol contributed to crystallization of hesperetin by acting as plasticizer, crystallization inducer and by providing heterogeneous nucleation sites. Hesperetin was found to exist as nanocrystals dispersed in the matrix of mannitol with average crystallite size of 137 nm in the NSD. [14]

NANOCRYSTAL STABILIZERS
The number of impressive advantages of nanocrystals, small size of nanocrystals can often lead to stability concerns. The large surface area of nanocrystals results in sufficiently high free energy or surface charge that might cause attraction or agglomeration. [15] Small sized Nanocrystals sometimes raise the solubility of drug beyond the saturation point which promotes recrystallization into larger particles; also known as Ostwald ripening. These processes ultimately lead to irreversible loss of formulation integrity. [16] There are number of stabilizers are used for stabilization of Nanocrystals

a. Poloxamer
Poloxamer are amphiphilic block copolymers formed with a combination of ethylene oxide (E; hydrophilic) and propylene oxide (P; hydrophobic) units arranged in an E–P–E arrangement. Poloxamers are available in various grades developed using different lengths of polymer blocks. They not only serve as ideal stabilizers but also presume the capacity to chemosensitize the multiple drug resistance (MDR) cells. Poloxamers are certified as generally recognized as safe (GRAS) excipient and considerably cause negligible hemolytic reaction hence they are popular for delivery of drugs through intravenous route. [19] They have been widely used for the stabilization of nanocrystals. Poloxamer 188 appended omeprazole nanocrystals showed enhanced stability due to shielding of the compound and on comparison with omeprazole solution, Researchers have also utilized Poloxamer 407 for the development of several nanocrystals formulations. Deng et al. attempted to improve the therapeutic profile of paclitaxel by stabilizing its nanocrystals using poloxamer 407, but failed, and ended up with thermosensitive micellar structure. However, renanonization with incubation sonication led to formation of nanocrystals with prolonged stability. [17,18]

b. Polyvinyl Pyrrolidone (PVP)
PVP or povidone is prepared by reaction of acetylene and pyrrolidone to form vinyl pyrrolidone followed by polymerization to convert into PVP. It is available in different viscosity grades having a versatile range of application from being a binder in tablets and capsules, film formers in ophthalmic solution, taste masking agent, toxicity reducer and the most important as a stabilizer in suspensions. [19] PVP K30 has been applied as a stabilizer for formation of celecoxib Nanocrystals Remarkably, it was seen that combination of stabilizers did not affect the crystallinity of drug when characterized by DSC; however a reduction of melting point was seen due to generation of new crystalline state. [21] PVP K17 and K12 demonstrated the versatile applications of PVP when they were tried for the preparation of probucol nanocrystals. The study established the fact that PVP or SDS alone was incapable to prevent agglomeration whereas combination of both resulted in a stable formulation. [20]

c. Polyvinyl alcohol (PVA)
Properties of the water soluble PVA are dependent upon the degree of polymerization and extent of hydrolysis. Partially hydrolysed PVA is generally used in pharmaceutical industry. [22] It has been used in formulating stable nitrendipine (a class II calcium channel blocker) nanocrystals through precipitation ultra-sonication method with resultant improved dissolution characteristics which in turn increased its oral bioavailability. [23]

d. Amino acid derived co-polymers
Albumin, a single polypeptide chain of 585 amino acids, is generally used as stabilizing agent for parenteral formulations containing protein and enzyme. Leucine (C6H13NO2) has gained usage as a lubricant and an antiadherent in the development of aqueous nanocrystals formulation. Lee et al., tried combination of various co-polymers derived from amino acids to stabilize nanocrystals consisting of naproxen. Nanoformulations were developed using two polymeric
combinations made of lysine, leucine, and albumin. Out of these two combinations its use is often associated with anaphylactic hypersensitivity reactions, hyperlipidemia, abnormal lipoprotein patterns, aggregation of erythrocytes and peripheral neuropathy.[24,26]

e. Lecithin
Lecithin is mixture of phosphatides with triglycerides, fatty acids and carbohydrates. Due to their lipid content, they form an integral part of many nutritional formulations. When used in pharmaceutical industry, they excel as stabilizer or emulsifiers. Their physical forms may vary from being powders or semi liquids based on their free fatty acid content and they also pose good absorption enhancing property. Being derived from natural sources (egg and soya) they find wide acceptance as stabilizer for a variety of drugs. Lecithin was used in combination with Poloxamer 188 and HPMC to stabilize amoiteone B, an anticancer agent. Yang et al., have employed dipalmitoyl phosphatidylcholine (a lecithin; endogenous component of human lung surfactant) to formulate nebulized itraconazole nanocrystals with improved bioavailability. The presence of dipalmitoyl phosphatidylcholine ultimately improved the overall in-vivo presence of itraconazole due to its permeation enhancement property.

f. Brij
It is used as emulsifying, wetting and a permeation enhancing agent, Brij-78 is a non-ionic surfactant containing Polyoxyethylene alkyl ethers and also termed as Cremophor. Nanocrystals obtained by processing oridonin were stabilized using Brij-78 by Gao et al. However, its use is often associated with anaphylactic hypersensitivity reactions, hyperlipidemia, abnormal lipoprotein patterns, aggregation of erythrocytes and peripheral neuropathy.[26]

g. Polysorbate 80
A Polyoxylene sorbitan fatty acid ester derivative has established itself as an important pharmaceutical excipient. They are classified according to type of fatty acid moiety which influences their functions. It has been utilized on the large scale as surface active agent however its propensity towards causing hypersensitivity, birth weight reduction in infants and other side effects when used in high concentration can sometimes be a deterrent. Therefore researchers often tend to utilize Polysorbate 80 at low levels as a complimentary stabilizer. It has been tried in combination with poloxomer 188, PVA, PVP and SDS for developing fenofibrate nanocrystals.[27]

h. Sodium laurel sulphate
A sulphuric acid monododecyl ester sodium salt, is an anionic surfactant used widely as a wetting agent however it exerts moderate toxic effects including irritation of the eyes, skin and stomach. It has been used to developed nanocrystals of herpetrione, an antiviral agent extracted from herpetospermum caudigerum, via high pressure homogenization technique followed by freeze drying.

i. HPMC
It is a widely used GRAS listed pharmaceutical excipient that has found special use in formulation of nanocrystals and is believed to cover the surface of crystal efficiently providing sufficient stability. In comparison to other stabilizers, its relatively high melting point makes it a robust option for production methods which involve high processing temperature. Recently, Ali et al., utilized HPMC as a stabilizing agent in media milling process of hydrocortisone nanocrystals intended for ophthalmic delivery. HPMC played a key role in stabilizing the nanocrystals by completely covering the surface of dried particle and gave it a low zeta potential. Figueroa et al., prepared HPMC based fenofibrate, naproxen, and griseofulvin (BCS class-II drug) nanocrystals. Inferences showed that HPMC was able to maintain the crystallinity of the bioactive. Hecq et al., used HPMC for producing nanocrystals of nimodipine. It using high pressure homogenization. The technology was utilized with the aim of formulating nanocrystals to improve the dissolution rate of nifedipine. The use of low viscosity grade HPMC provided adequate stabilizing effect compared to other surface active agents like SDS, poloxamer and polysorbate.[28,29]

j. Sodium cholic acid
Bile acid derived white crystalline powder is employed to stabilize many nanocrystals based formulations. In combination with poloxomer 188, it has been used to stabilize nimodipine. It nanocrystals. Nimodipine. It is regarded as a drug of choice for reducing both morbidity and mortality in subarachnoid haemorrhage related vasospasm. The clinically available injectable form of the drug is administered with alcohol and PEG 4000 which is associated with numerous allergic reactions. The sodium cholic acid stabilized nanocrystals thus
offered a novel approach in which the excipients did not adversely affect the risk–benefit ratio and open an option for intravenous administration of nimodipine. It has also been used for stabilization of cyclosporine nanocrystals. \[30\]

**APPLICATION OF NANOCRYSTALS:**

1. **Oral drug delivery**

   Oral route has been the most preferred route and is considered as the safest and suitable route for drug delivery.\[31,32\] For orally administered drugs, dissolution is considered as a rate determining step for absorption. Nanocrystals provide a greater surface area for dissolution and thus raising the saturation solubility which ultimately increases the dissolution rate thereby enhancing drug absorption. Muller et al., have refined oral delivery of thermo stable drugs utilizing melted PEG (melting point at 60 °C) which allows fixing of nanocrystals in a solid PEG matrix. Nanocrystals dispersed in melted PEG were milled to powder and directly compressed into the tablet or filled in capsules shell. Thus, this novel drug delivery system offers a way to incorporate poorly soluble drugs directly into tablet, capsule or hot melts solid matrix to improve oral bioavailability.\[33\]

2. **Intravenous drug delivery**

   Administering a drug via intravenous route provides numerous benefits such as immediate action, reduced dosing and 100% bioavailability. The use of intravenous route is limited because harmful solvent and excipients, which are used during formulation development, are also co-administered with the drug and they can cause serious side effects other than the drug itself. Nanocrystals could be considered as the ideal candidates for intravenous delivery because their developmental processes do not employ excess use of such harmful excipients.\[33\]

3. **Pulmonary drug delivery**

   Lungs are highly perfused organs with a fully expanded surface area roughly equivalent to three football fields. Due to lack of hepatic portal drainage, molecular dispersion of drug is rapidly transported into the systemic circulation with high efficiency. Recently, it has been demonstrated that pulmonary nanocrystals have the ability to rival pharmacokinetics offered by intravenous administration of baicalin. Pulmonary route thus comes across as a viable option for delivery of therapeutics. Due to constant exposure to external environment, it is highly susceptible to disease causing agents; allergens and pathogens easily invade through the respiratory tract. Conventional modalities of deep lung drug deposition have been modified by tailoring size of nanocrystals. A nebulizer is generally required to administer powdered nanocrystals. Nebulizer can incorporate nanocrystals into small inhalable droplets (1–5 μm).\[33,34\]

4. **Ocular drug delivery**

   Ophthalmic drug delivery is a challenging task owing to critical pharmacokinetic environment and physiological barriers of the eye that hinder the delivery of drugs. Most of the drugs for ocular therapy are delivered through a topical formulation in the form of solution or suspension. Conventional formulations are subjected to rapid clearance from application site due to rapid eye movements (blinking) and lacrimation, which results in low ocular availability. Short retention time of medication induces a need of repeated dosing relatable with loss in patient compliance and dose dependent side effects. To alleviate these rapid filings, several approaches like ocular inserts and ophthalmic gels have been tried, which themselves are associated with fair share of inadequacies, viz. poor therapeutic outcome, blurred vision and local irritation. Ophthalmic drug delivery was believed to be benefited largely by a colloidal drug delivery system. Piloplex, the first novel colloidal drug delivery system developed, contains pilocarpine which is ionically bounded to poly (methyl) methacrylate-co acrylic acid nanoparticles.

   Subsequently, nanocrystals technology played an advanced role in ophthalmic drug delivery tackling dispersibility issues of poorly soluble drugs such as budesonide, dexamethasone, hydrocortisone prednisolone and fluorometholone. Ali et al., used combination technology based upon microfluidic nanoprecipitation and wet milling to create nanocrystals of hydrocortisone and ocular bioavailability was evaluated in albino rabbits. Results demonstrated an extended duration of action and significantly improved AUC of developed nanocrystals in comparison to free drug. A markedly advanced ophthalmic delivery system for forskolin (intra ocular pressure lowering agent) was developed by incorporating its nanocrystals into an in-situ gelling system comprised of poloxamer and polycarbophil. Pharmacodynamics studies revealed that nanocrystals/hydrogel system efficiently lowered the intraocular pressure up to 12 hrs in comparison to conventional suspension.
5. Bioavailability Enhancement
Some of newly developed molecules having the problem of poor water solubility so resulting poor permeability. Nanosuspensions resolve the problem of poor bioavailability by solving the twin problems of poor solubility and poor permeability across the membrane. As compared to conventional naproxen the oral administration of naproxen nanoparticles lead to an area under the curve (AUC) (0-24 h) of 97.5 mg-h/l which is just 44.7 mg-h/l for naproxen suspensions and 32.7 mg-h/l for anaprox tablets. The Oral administration of the gonadotropin inhibitor in conventional dispersion (Danocrine) only shows 5.2% absolute bioavailability but in the form of Nanosuspension Danazol it’s about 82.3%. Kayser et al. developed the Nanosuspension Amphotericin B showed a significant improvement in its oral absorption in comparison with the conventional commercial formulation.[13]

6. Targeted Drug Delivery
Nanosuspensions can be used for targeted delivery as their surface properties and in-vivo behavior can easily be altered by changing either the stabilizer or the milieu. The engineering of stealth Nanoosuspensions (analogous to stealth liposomes) by using various surface coatings for active or passive targeting of the desired site is the future of targeted drug delivery systems. Kayser et al formulated a nanosuspension of Aphidicolin to improve drug targeting against leishmania infected macrophages. He stated that nanosuspension formulation had an enhanced activity with an EC (50) of 0.003 mcg/ml which is about 0.16mcg/ml in the conventional form. Scholer et al. showed an improved drug targeting to the brain in the treatment of toxoplastic encephalitis in a new murine model infected with Toxoplasma gondii using a nanosuspension formulation of Atovaquone.[13]

MARKETED FORMULATION OF NANOCRYSTALS
1. Rapamune
It was the first US FDA approved oral nanocrystals launched in the year 2000 by Wyeth Pharmaceuticals (Madison, NJ). It consists of Sirolimus nanocrystals incorporated in an excipient mixture suited for direct compression into palatable tablets. The oral bioavailability of the nanocrystals tablets was 21% higher compared to Sirolimus solution. Previously Rapamune available only as an oral solution which requires refrigeration storage and mixed with water and orange juice prior to administration.[35]

2. Emend
It was introduced market in 2001 by Merck (Winehouse Station, NJ), it consists of Aprepitant, which is generally used for treatment of emesis. Aprepitant is a selective, high-affinity antagonist of human substance P/neurokinin 1 (NK 1) receptors. Aprepitant has little or no affinity for serotonin (5-HT 3), dopamine, and corticosteroid receptors, the targets of existing therapies for chemotherapy-induced nausea and vomiting (CINV). Aprepitant has been shown in animal models to inhibit emesis induced by cytotoxic chemotherapeutic agents, such as cisplatin, via central actions. Emend is capsule containing 80 or 125 mg of Aprepitant formulated as Nanocrystals drug particles.[36]

3. Tricor
It was introduced in market by Abbott Laboratories and the active ingredient is fenofibrate, being available in 48 mg and 145 mg tablets. Tricor is indicated as adjunctive therapy to diet in adult patients with primary hypercholesterolemia or mixed dyslipidemia (Fredrickson types IIa and IIb) to increase high-density lipoprotein cholesterol (HDL-C), reduce triglycerides (TG), reduce low-density lipoprotein cholesterol (LDL-C), reduce total cholesterol (Total-C), and reduce Apo lipoprotein B (Apo B).[37,38]

4. Megace ES
Megace Es (megesterol acetate) was introduced by Par Pharmaceutical Companies, Inc. (Spring Valley, NY), who licensed the Megace name from Bristol-Myers Squibb (New York). Megestrol is a synthetic progestin and has the same physiologic effects as natural progesterone. Megestrol also has direct cytotoxic effects on breast cancer cells in tissue culture and suppresses luteinizing hormone release from the pituitary. It is mainly used to improve weight gain and appetite in patients undergoing chemotherapy or suffering from an HIV infection. The Nanosized drug can be formulated in less volume, so the single dose the patient has to take (daily dose 625 mg of Megestrol in 5 ml of fluid) is reduced by the factor four compared to the oral solution available. This reduced volume and the improved bioavailability lead to a better patient compliance due to the possibility of flexible dosing in order to provide effective appetite stimulation and weight gain.[2]
CONCLUSION

Drug Nanocrystals are considered as the most important formulation approaches for poorly water soluble drugs. This technology can be applied to any poorly water soluble drugs to overcome the solubility and bioavailability problems. The decrease in the particle size in the Nano range. Solubility enhancement alone is not the only important factor; rather it becomes even more important when a drug has a narrow therapeutic window where it can be absorbed. In these cases the increased solubility and dissolution velocity lead to an acceptable bioavailability. This technology enables formulations to be developed without the need of problematic surfactants (e.g. Cremophor EL) which may cause enhanced side effects or adverse reactions. Nanocrystals also allow for a fast action onset, as the drug is absorbed quickly due to the fast dissolution of the nanoparticles. This is an advantage, especially for drugs which need to work fast (e.g., naproxen for headache relief). By modifying the nanocrystals surface it is possible to achieve a prolonged or a targeted release. Drug nanocrystals is that it can provide smaller dose administration to achieve moderate blood level and thus reduce the side effect from given larger dosage. It can be applied to various administration routes such as oral, parenteral, ocular, pulmonary and dermal delivery. The liquid Nanosuspensions can be employed as a liquid dosage form or transformed into solid dry powder for further production of tablets, capsules, or pellets dosage forms. Several techniques can be used to solidify the Nanosuspension including the preparation as the granulation fluid for tablet production, the layering dispersion in fluidized bed process, the use of solid/liquid PEG, spray drying and lyophilization. By application of these technology produce final dosage forms with higher drug loading capacity, better redispersibility at their site of action, as well as an improved drug targeting.

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