Nanocrystals in Drug Delivery – A Review

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Abstract **- Nanocrystals are the aggregates anywhere from a few hundred to tens of thousands of atoms that combine into a crystalline form of matter known as a " Cluster " .They are used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. They have been used in vivo to protect the drug entity in the systemic circulation. The method of preparations of nanocrystal is down, top down and bottom up, spray drying and so new techniques. There are several important advantages of nanocrystal formulation such as, enhanced oral bioavailability, improved dose proportionality, reduced food effects, suitability for administration by all routes and possibility of sterile filtration due to decreased particle size range. Selection depends upon the sites and to deliver the drug at a controlled sustained rate to the site of action. Here we review various aspects of nanocrystals in drug delivery and their pharmaceutical applications in drug delivery.**

Index Terms **- Nanocrystal, Drug delivery, Bioavailability, polymeric nanocrystals, techniques.**

1.INTRODUCTION

Drug nanocrystal are pure solid drug particles with a mean diameter below1000nm. Drug nanocrystals are the nanoparticles which offer an advantage of 100% drug loading since they are encapsulating-carrier free nanoparticles. Nanocrystals formulation contains drug and one/more stabilizers dispersed in aqueous or non-aqueous media. Stabilizers could be one or more generally regarded as safe excipients (surfactants or buffers, salts or sugars). The liquid dispersion nanocrystals could be further post processed into solid or sterile injectable dosage forms. The therapeutic applications of nanocrystals products have been identified in oral, parenteral, ocular, dermal, pulmonary, and targeted drug delivery. Contrary to micronized drugs, nanocrystals can be administered via several routes. Oral administration is possible in the form of tablets, capsules, sachets or powder; preferably in the form of a tablet. Nano suspensions can also be administered via the intravenous route due to very small particle size, and in this way, bioavailability can reach 100 %.

2.PREPARATION OF NANOCRYSTALS

Properties such as crystallinity, size, shape, surface charge, and the type of stabilizers or polymer coatings used during formulation Influence the therapeutic outcome of nanocrystal drug products. Other physicochemical properties currently under investigation for their influence on preclinical (i.e., in vivo/in vitro) performance assays Include stiffness and surface texture.

Drug nanocrystals contained almost 100% drug and only small amounts of stabilizers, which anchoring on the surface of as-prepared drug nanocrystals through ionic or steric stabilization. It is important to select suitable drugs and specific stabilizers in the preparation process of drug Nanocrystals. Generally, poorly-water soluble or lipophilic drugs are selected and stabilized. The commonly used stabilizers mainly contain: (1) polymers, such as polyvinyl pyrrolidone (PVP), hydroxypropyl methyl cellulose (HPMC), and hydroxypropyl cellulose (HPC); (2) ionic surfactants, such as sodium dodecyl sulfate (SDS); (3) non-ionic surfactants, such as tweens and poloxamers (polyoxyethylene–polyoxypropylene copolymers). The interaction forces between stabilizers and drug nanocrystals are different, for instance, ionic surfactants stabilizing drug nanocrystals via electrostatic repulsion, while polymers and non-ionic surfactants coating drug nanocrystals via steric repulsion. (1)There are three main synthetic methods of drug nanocrystals, including top-down (size

reduction of large drug particles), bottom-up (nucleation and growth), and combination approaches.

VARIOUS METHODS OF NANOCRYSTAL PREPARATION

There are various methods developed to prepare drug nanocrystals. The milling processes include disintegration and homogenization by the use of mechanical forces to disintegrate active pharmaceutical ingredients into nanosized particles. Using this method, there are a number of commercial products available in the market and has been approved by regulatory bodies. However, these types of methods utilize high energy, or pressure to produce nanoscale size. In addition, mechanical attrition leads to some associated drawbacks such as high energy use, time-consuming and no control on particle size, and electrostatic effects.

In the crystallization method of preparation, there is minimum mechanical energy used to prepare the nanocrystals. The crystallization method involves the following steps: (1) dissolution; (2) nucleation; (3) growth of the crystals; and (4) filtration followed by drying. Furthermore, various crystallization techniques such as supercritical fluid, high gravity, cryogenic techniques, ultrasonication, and microemulsion methods were used to produce the nanocrystals.

Several preparation methods devolved today, implemented preparation methods of nanocrystal formulations can be classified as “bottom up”, “top-down”, “top down and bottom up” and “spray drying”. “Bottom Up” technology begins with the molecule; active drug substance is dissolved by adding an organic solvent, and then, solvent is removed by precipitation. “Top down “technology applies dispersing methods by using different types of milling and homogenization techniques. “Top down” technology is more popular than “bottom up” technology; it is known as “nanosizing”. In other words, it is a process which breaks down large crystalline particles into small pieces. In “top down and bottom up” technology, both methods are utilized together. Spray drying is also a method for preparing drug nanocrystals which is faster and more practical compared to the other Methods.

1. Bottom up
a) Nano precipitation
2. Top down
a) Milling
b) Homogenization
3. Top down and Bottom up
4. Spray drying
5. Other Techniques used for the Production of Drug Nanocrystals
a) Rapid expansion from a liquefied-gas solution (RESS)
b) Nanopure® XP technology
c) Spray Freezing into Liquid (SFL) technology.

a. Bottom up technology

The principle of this method is based on the dissolution of the active drug substance in an organic solvent which is then added into a non-solvent (miscible with the organic solvent). In the presence of stabilizers, thereafter, the nanocrystals are precipitated. Basic advantage of the precipitation technique is that it is simple and has a low cost. Also, scale up is simple in this method. It should be kept in mind that several parameters; such as stirring rate, temperature, solvent/ nonsolvent rate, drug concentration, viscosity, type of solvent and stabilizer should be controlled in order to obtain homogenous nano crystals by this technique.

b. Top down technology

“Top-down” technology applies dispersing methods by using different types of milling and Homogenization techniques. “Top-down” technology is more popular than “Bottom up” technology; it is known as “nanosizing”. In other words, it is a process which breaks down large crystalline particles into small pieces. In “top Down and bottom up” technology, both methods are utilized together. Top-down technology can be applied by either homogenization or milling. Top-down approach refers to the particle size reduction of coarse drugs down to nanoscale drug crystals by milling or high-pressure homogenization (HPH) methods. In milling method, four forces, such as shearing, attrition, impact, or pressure, are involved to pulverize the coarse drugs, and wet ball milling (bead or pearl milling) is the most frequently used method in the pharmaceutical industry. This method will generally yield well-defined drug nanocrystals with a narrow size distribution after enough time of milling. Besides milling, the HPH technique is using jet-stream homogenization of drugs, dispersion medium,

surfactants, and/or stabilizers under high pressure through a very thin gap (typically about 25 μm) at an extremely high velocity. The particle size reduction of drug nanocrystals, caused by cavitation forces, shear forces and collision during multiple homogenization cycles, is dependent on many factors such as the types of homogenizers, pressures, and cycles. In HPH method, the size distribution of drug nanocrystals is strongly dependent on the brittleness, hardness, and defect density of the initial drug crystals.

Besides milling and HPH methods, laser fragmentation was also used to prepare drug Nanocrystals by focusing a femtosecond or nanosecond laser radiation on a magnetically stirred drug suspension in water or aqueous solution of a stabilizing agent. For example, PTX43 and megestrol acetate (MA) have been fragmented into nanocrystals by using this method. For the synthesis of MA nanocrystals, the femtosecond laser fragmentation was performed with vertical configuration, and the nanosecond laser fragmentation was performed with horizontal configuration. Compare with untreated water-exposed, ~30% and ~60% of the MA mass was <1 μm after the femtosecond and nanosecond laser treatments, respectively. The scanning electron microscope (SEM) observation is in agreement with size distribution analysis by dynamic light scattering (DLS) in. Besides the high energy input and high risk of contamination, both of above top-down methods cannot produce small size drug nanocrystals (< 100 nm). Therefore, other methods are actively developed to address these issues.

c. Top down and bottom up technology

In "top down and bottom up" technology, both Methods are used together. Nano-Edge® is a product Obtained by such a combination technology. Nano-edge Technology described the formulation method for poorly Water-soluble drugs. It is a useful technology for active Ingredients that have high melting points and high Noctanol-water partition coefficients. It is based on direct Homogenization, micro precipitation, and lipid emulsions. In micro precipitation, the drug first is dissolved in a Water-miscible solvent to form a solution. Then, the Solution is mixed with a second solvent to form a pre-Suspension and energy is added to the pre suspension to Form particles having an average effective particle size of 400 nm to 2 μ.

d. Spray Drying

One of the preparation methods of nanocrystals is 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.

e. Combination Methods

To obtain small (< 100 nm) and narrow size distribution of drug nanocrystals and overcome drawbacks of long production times, top-down and bottom-up methods were combined. Combination techniques have also been developed that integrate a pre-treatment step with a subsequent high energy step, like HPH. Nowadays, bottom-up method was firstly employed as a pre-treatment and followed by top-down method for further homogenization, such as NANOEDGE54 (micro-precipitation followed by HPH), H 69 (micro-precipitation immediately followed by HPH, also called 'cavi-precipitation'), and the combination technology (media milling followed by HPH). Due to the Ostwald ripening and settling, nanosuspensions containing nanocrystals are not stable in liquid environment, therefore, spray-drying and freeze-drying methods are employed for further solidification, such as H 42 (spray-drying followed by HPH) and H 96 (freeze-drying followed by HPH). As anticancer drugs including PTX, hydroxycamptothecin (10-HCPT), and cyclosporine were formulated into drug nanocrystals by combination methods.

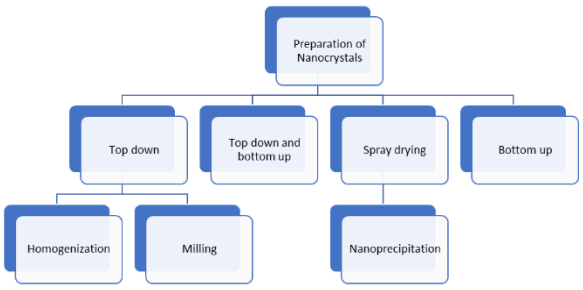

Fig. 1: Methods of nanocrystal preparation

3.CHARACTERIZATION OF NANOCRYSTALS

For the successful fabrication of a nanocrystal formulation, besides selection of the appropriate excipients, equally important is the characterization of the formulation to ensure that the necessary parameters responsible for the performance of nanocrystals are within the specified limits. The following sections discuss in detail the various characterization tests for the evaluation of nanocrystals.

3.1. Solid State Properties

The solid state properties (polymorphic crystal form, solvate (especially hydrate) form, degree of crystallinity) influences the apparent solubility and thereby the dissolution rate. Hence, it is crucial to determine these characteristics in nanocrystals. Ideally, thermodynamically most stable crystalline form is desirable to prevent the peril of solid state transformations during production, storage and/or administration.

Different nanocrystal manufacturing conditions and procedures can have an impact on the ensuing solid state form. Furthermore, the environmental conditions affect the thermodynamically stable polymorphic form. For e.g., hydrate forms are generally more stable (and therefore less soluble) in aqueous media.(2)

3.2. Thermal Analysis

Differential scanning calorimetry (DSC) is one recurrently used method for studying the thermal behavior of drug and drug nanocrystals. The DSC studies are performed to check the status of crystallinity of drug and interaction of excipients and drug after production of nanocrystals. This is especially important for drugs occurring in different polymorphic forms. Moreover, certain top-down techniques like the high pressure homogenization can lead to particles with an amorphous fraction, thus leading to enhancement of saturation solubility.(2)

The furnace is heated at a linear heating rate, and the heat is transferred to the sample and reference pan through the thermoelectric disk. However, owing to the heat capacity (Cp) of the sample, there would be a discrepancy in the temperature between the sample and reference pans, which is measured by area thermocouples, and the consequent heat flow is determined by the thermal equivalent of Ohm's law: $q = \Delta T/R$ where q is "sample heat flow", T is "temperature difference between sample and reference", and R is "resistance of thermoelectric disk". (3)In a power-compensated DSC, the sample and reference pans are placed in separate furnaces heated by separate heaters. The sample and reference pans are maintained at the same temperature, and the difference in thermal power required to maintain them at the same temperature is determined and plotted against temperature or time.(3,4)

3.3. X-ray Diffraction (XRD)

X-ray diffraction studies are usually performed for the confirmation of drug crystallinity following its conversion to a nanocrystal formulation. When X-rays interact with a crystalline substance, a diffraction pattern is obtained. Every crystalline substance gives a specific pattern; the same substance always yields the same pattern; and in a mixture of substances each produces its pattern independently of the others. The X-ray diffraction pattern of a substance therefore represents the unique fingerprint of the substance. The authors carried out XRD to analyze the modification in the crystalline nature of the drug following its conversion into nanocrystals.

In addition, the powder XRD study of spray dried nanosuspension prepared by top-down process (high speed milling) showed negligible shift in the main peaks as compared to pure drug. The characteristic peaks for milled and unmilled drug were observed at the same 2θ values. A slight decrease in intensity of peaks was observed with spray dried nanosuspension operated at higher milling speed. (5)

3.4. FT-IR Studies

Chemical properties of drug and interaction with excipients are evaluated by FT-IR studies. Liandong et

al. formulated and evaluated curcumin nanocrystals for pulmonary delivery. FTIR studies of the pure drug and the developed dry powder inhalation (wet-milling followed by spray-drying) were done for evaluation of change in chemical properties of the drug.(6)

3.5. Raman Spectroscopy

Raman spectroscopy is a spectroscopic technique based on inelastic scattering of monochromatic light, originating from a laser source. Inelastic scattering means that the frequency of photons in monochromatic light amends following interaction with a sample. Photons of the laser light are absorbed by the sample and then reemitted. Frequency of the reemitted photons is shifted up or down compared to that from the original monochromatic frequency.

The size of nanocrystals in this process was influenced by factors such as the freezing rate. Hence, to determine during what stage of the process the solutes crystallized and how the freezing rate impacted the particle size, the crystallization process was monitored by Raman Spectroscopy. (7)

Liquid atomization-based techniques, like spray drying or electrospraying, are markedly susceptible to generating a final product in the amorphous form (partially or fully). However, full crystallinity can be obtained after production by annealing. The high shear stresses associated with wet media milling and high-pressure homogenization may also result in polymorphic changes. (8)

Nanocrystals were produced by wet ball milling, with poloxamer 188 used as a stabilizer. There were no significant differences between the particle size of the two polymorphs when the same milling protocol was used, but differences in the stability with respect to the particle size were seen during the 90 days of stability testing. The milling did not alter the polymorphic form of the drug. The crystallite size of the milled polymorphs was calculated based on XRPD peak width broadening. It was observed that for polymorph 1, the crystallite size was around 90 nm while for polymorph 2 it was around 65 nm.(9)

3.6. Particle Size and Size Distribution

Size and size distribution are important characterizations of the nanosuspensions because they direct the other properties, such as physical stability, saturation solubility and dissolution velocity, and even clinical efficacy. The smaller the particle size, the higher the surface energy of the particles, which promotes aggregation. The most frequently used techniques for particle size measurements of nanosized systems are dynamic light scattering techniques, static light scattering techniques and microscopy.

The polydispersity index (PI) value ranges from 0(monodisperse particles) to 0.500 (broad distribution), and is a crucial index that governs the physical stability. For a long-term stability the PI should be as low as possible. Techniques for the detection of larger particles are optical microscopy and low angle static light scattering (laser light diffraction), especially for the nanosuspensions that are meant for parenteral and pulmonary delivery.

The Laser diffractometry (LD) yields a volume distribution and possesses a measuring range of approximately 0.05–80 μm up to a maximum of 2000 μm, depending on the type of equipment employed. Typical characterization parameters of LD are diameters 50%, 90%, 99%, represented by D50, D90, and D99, respectively (i.e., the D50 implies that 50% of the volume of the particles is below the given size). The disadvantages of laser diffraction techniques rose with the need of analyzing nanoparticles with a technique being originally intended for the measurement of larger particles in the micron range. Since laser diffraction is a simple and rapid method it was aimed to extend the measuring range (e.g., from 400 nm to 2000 m) to a broader range, being able to analyze even very small particles (e.g., from 20 nm to 2000 m) py, and scanning tunnelling microscopy. (10)

3.7. Particle Shape and Morphology

Ideally, the shape or morphology of the nanocrystals can be determined using a transmission electron microscope (TEM) and/or a scanning electron microscope (SEM). A wet sample of suitable concentration is needed for the TEM analysis. When the formulated nanosuspensions are to be converted into a dried powder (e.g., by spray drying or lyophilization), a SEM analysis is crucial to monitor alterations in the particle shape and size before and following the process of the water removal. (11)

Atomic force microscopy (AFM), a kind of scanning probe microscope is designed to measure local properties, such as height, friction, magnetism with a probe. (12)

Surface plasmon resonance (SPR) analysis has been employed in interaction studies between solid drug surfaces and aqueous stabilizer solutions. Five structurally different PPO/PEO block co-polymers were used as stabilizers for indomethacin nanocrystals, and affinities between the stabilizers and solid drug surfaces were determined by SPR and contact angle measurements. (13)

Particle shape is of prime importance when the nanocrystals are to be formulated as dry powder inhalers (DPIs) for direct lung delivery of the drugs.

Particle interactions are linked to the van der Waals forces, which are the particle surface morphology, size, shape, electrostatic properties and hygroscopicity. Particle shape that possess low contact area and van der Waals force have a lower tendency to aggregate and hence can be readily dispersed in the air. Elongated particles are not ideal for aerosolization owing to their large attractive forces.(14)

3.8. Particle Surface Charge

The surface charge of the particles is one of the factors influencing the physical stability of nanosuspensions. The higher the particles are equally charged, greater is the electrostatic repulsion between the particles and greater is the physical stability. The particle surface charge is ideally quantified in terms of the "zeta potential", which is measured via the electrophoretic mobility of the particles in an electric field. The particle charge can be measured in surface charge per unit, determined by colloid titration. (12,13)

In an electrolyte containing media, ions from the dispersion medium adsorb onto the particle surface. For this model description a negative Nernst potential is assumed. In general, the first absorbed monolayer of ions comprises of negatively charged, fixed and dehydrated ions, termed as the Helmholtz layer.

The measurement itself is a particle electrophoresis, the particle velocity is determined via the Doppler shift of the laser light scattered by the moving particles.

The field strength applied is generally 20 V/cm. The electrophoretic mobility was converted to the zeta potential in mV using the Helmholtz–Smoluchowski equation. At standard measuring conditions (room temperature of 25 ◦C, water) this equation can be simplified to the multiplication of the measured electrophoretic mobility (µm/cm per V/cm) by a factor of 12.8, yielding the ZP in mV. (14,15)

3.9. Dissolution of Nanocrystals

Apparent Solubility and Supersaturated State

Thermodynamic solubility implies the solubility of the most stable crystalline form of the drug in a given medium at a specified pressure and temperature. Solubility can temporarily be higher than the thermodynamic solubility. This may be observed with amorphous forms, metastable polymorphic forms, or nanosized drug particles. This enhanced solubility has been designated with diverse terms, such as kinetic or apparent solubility. (16)

The thermodynamic solubility of the bulk drug in aqueous 0.5% and 1% sodium dodecyl sulfate solution was 6.02 and 23.54 µg/mL, respectively, while the corresponding values for drug nanocrystals were 67.51 and 107 µg/mL, respectively. (17)

The intrinsic dissolution rates were profoundly influenced by the particle size and the stabilizer. With the smallest nanocrystals (580 nm), the intrinsic dissolution rate with poloxamer F68 as a stabilizer was 0.50 µg/min/mm^2, while that for poloxamer F127 was 0.31 µg/min/mm^2. The dissolution rate of bulk indomethacin was also determined and found to be considerably lower at 0.05 µg/min/mm^2. Surface concentrations have also been measured with UV-imaging.

Indomethacin, the model drug used was found to interact with both the type of filter tested as well as the centrifuge tube material. Undissolved drug particles in the sample can be recognized employing multiple wavelengths for the analysis.(18)

The parachute effect of the polymer can be due to a combination of mechanisms. First, the polymers can themselves increase the thermodynamic solubility of the drug (also termed as the co-solvency effect), which lowers supersaturation and consequently the thermodynamic driving force for crystallization (this also leads to an additional spring effect with the polymer). Through drug-polymer interaction in solution via electrostatic bonds, van der Waals' forces or hydrogen bonding, even the addition of small amounts of polymers such as PVP and HPMC to solution can significantly increase the aqueous solubility.(14)

3.10. Permeation Study

Nanocrystal based drug delivery could be very effective for improving dermal bioavailability of drugs with poor solubility. Indeed, in addition to increased

saturation solubility and dissolution rate, nanocrystal also exhibits the property of increased adhesiveness to the skin thus facilitating the dermal delivery. The two mechanisms by which drug is delivered to the skin; first one is simple increase of concentration gradient between formulation and skin and the second mechanism involves hair follicles. Nanocrystals with an appropriate size (approximately 700 nm) can deposit into these shunts, which act as a depot from which the drug can diffuse into the surrounding cells for extended release(19,20)

The nanocrystal based drug delivery to the eye can be exploited for improving retention and penetration of drug in to the eye. The possible mechanism for this is not only to increase solubility in lachrymal fluid but also to produce adhesive properties. Nanocrystals may be used not only to increase solubility in lachrymal fluids of poorly soluble drugs, but also to produce adhesive properties (determined by the nature of the surfactant in the formulation) that can be exploited for improving the retention and penetration of drugs into the eye. Non-ionic surfactants are preferred over ionic because they are generally less irritating. The permeation studies are usually done by using the Franz diffusion cell apparatus (21,22)

3.11. Drug Absorption from Nanocrystalline Formulations

Drug absorption is directly related to both solubility and permeability and inversely related to lipophilicity. Dissolution from nanocrystals is followed by permeation of the dissolved drug across the gastrointestinal wall (in the same way as drug from a solution formulation). Besides increasing permeation of the drug due to elevated dissolved concentrations, stabilizers present in the formulation themselves interact with cells of the epithelial layers to promote permeation.

They studied five different drugs and nanocrystals were formulated using the same stabilizer, poloxamer 188, by high pressure homogenization. Particle size obtained for all the tested drugs was almost 430 ± 30 nm. The AUC values in all the cases were 1.4–7.2 times higher as compared to drug microsuspensions following oral administration to rats. Melting point, log p value and polar surface area were found to have an influence on drug absorption. Drugs with low melting point, log p value approximately 5 and polar surface area value between 50 and 60 manifested higher absorption with the same sized nanocrystals.(23)

The potential of TPGS stabilized paclitaxel nanocrystals to reverse P-glycoprotein drug-resistance in P-gp overexpressing H460 cancer cells was evaluated by Gao with colleagues. It was found that TPGS as a stabilizer efficiently lowered drug resistance of the studied cells. It is known that due to the enhanced permeation and retention (EPR) effect, drug nanoparticles accumulate in the tumor tissues following an intravenous injection.

Confocal Raman microscopy and coherent anti-Stokes Raman scattering (CARS) microscopy are novel label-free, chemically specific and non-destructive methods with potential for label-free imaging of nanocrystal-cell interactions. With these techniques submicron particles may be analyzed provided they have a sufficiently strong Raman or CARS signal (the resolution and speed is better for the inherently confocal CARS technique, while chemical specificity is better for Raman microscopy).

The nanocrystals were imaged in both fixed and live cells using the CH2 stretching resonance at 2845 cm−1, mainly associated with the palmitate moiety (the nanocrystals were resolved from endogenous lipid in this case through geometrical differences, and an otherwise weak lipid signal from the cells was used, although with other drugs a CARS resonance resolved from lipid signals could be used for chemical specificity). In tissue sections, intracellular nanocrystals were imaged within the granulomatous tissue. (24,25)

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