Exploring Drug Nanocrystals: Comprehensive Insights Into Their Formulation And Characterization

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ABSTRACT

Poor drug solubility remains a significant barrier to the development of highly potent pharmaceutical agents. Drugs with low solubility often exhibit poor oral bioavailability and inconsistent absorption, a concern particularly relevant to Biopharmaceutical Classification System (BCS) Class II drugs. In this class, the primary limitation to drug absorption is the slow dissolution rate caused by low solubility. Despite their high permeability, these drugs suffer from a limited concentration gradient between the gastrointestinal tract and the bloodstream, ultimately hindering effective drug transport and oral absorption.

KEYWORDS Nano crystals, Solubility, BCS classification, Bioavailability.

INTRODUCTION

Nowadays, there are a large percentage of drug compounds in drug development represents as poor aqueous solubility. 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. Several strategies have been employed to overcome these limitations. 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 / pseudopolymorphs (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 [1-5].

Particle size reduction has been a much smarter approach that can be applied to nonspecific formulation for many years. The micronization of drug leads to an increase in their surface area which proportionally increases in rate of dissolution and rate of diffusion (absorption). However, for very low solubility compounds the micronization fails to improve the saturation solubility and increase the bioavailability of the drug. Therefore, the further step to reduce the particle dimension to nanometer size range has been invented. Recently, particle diminution to the sub-micron range has emerged to be a powerful formulation approach that can increase the dissolution rate and the saturation solubility, subsequently improve the bioavailability of poorly water-soluble drugs and may also decrease systemic side effects. Over the last decade, drug nano crystals are considered as a novel approach to improve the solubility of hydrophobic drugs since the technique is simple and effective which can quickly launch

product to the market. The nano crystals were invented at the beginning ofthe 1990s and the first products appeared very fast on the market from the year 2000 onwards. Additionally, drug nanocrystals are a universal approach generally applied to all poorly soluble drugs for the reason that all drugs can be disintegrated into nanometer-sized particles [6]. Drug nanocrystals are nanoscopic crystals of parent compounds with the dimension of less than 1 µm. They are composed of 100% drug without carriers and typically stabilized with surfactants or polymeric steric stabilizers. A dispersion of drug nanocrystals in an outer liquid medium and stabilized by surface active agents is so-called nanosuspensions. The dispersion medium can be water, aqueous or nonaqueous media e.g. liquid polyethylene glycol (PEG) and oils. The nanosuspensions can be used to formulate compounds that are insoluble in both water and oil and to reformulate existing drugs to remove toxicologically less favorable excipients. Additionally, the poorly soluble drugs enable to be formulated as nanosuspensions alone, or with a combination of pharmaceutical excipients [2,4,7].

The properties of drug nanocrystals that should be concerned and the given benefits over the micronized particles aresummarized as follows.

1. Particle size below 1 µm
2. 100% Drug, no carrier
3. Generally needed to be stabilized by surface active agent
4. Crystalline or amorphous structure(Amorphous state offering advantages)
5. Increase in saturation solubility
6 .Increase in dissolution velocity

PREPARATION OF DRUG NANOCRYSTALS

Several preparation methods for drug nanocrystals have been investigated. The techniques to produce drug nanocrystals can be divided in two basic approaches, namely the bottom up and the top down technologies. To obtain nanoparticles of drugs, the top down processes involve a breaking down of larger particles by milling or homogenization, while the bottom up processes associate with an assembling and controlling of precipitations at nanometer scale. An overview of drug nanocrystals for oral administration which were prepared by different techniques in current marketed and during pharmaceutical researches was shown in Table 1.

Bottom up processes[2,4,6,8]

Starting from the molecules in solution, the molecules are aggregated to form particles that can be crystalline or amorphous form. This technique may be called 'a classical precipitation process' (in latin: via humidaparatum). In this technique, the drug is completely dissolved in a solvent. Then the solvent solution is added to a non-solvent, causing precipitation of the drug. Importantly, it is necessary to control the structure of the particles and to avoid the growth of the particles to the micrometer size range by controlling influence factors and adding stabilizers such as surfactants. Other bottom up technologies include sonocrystallization, the high gravity controlled precipitation technology, confined impinging liquid jet precipitation and multi-inlet vortex mixing.Bottom up processes open the ways of interesting possibilities to incorporate multiple active ingredients in a single nanocarrier and to tailor nanoparticle surface functionality.However, a basic disadvantage of many precipitation processes is the use of organic solvent which is needed to be removed, leading to the high cost of production. Particularly,in case of low water and organic solvent soluble drug, the large solvent volumes are required. Hence, in pharmaceutical industry,the bottom up processes has not been employed for the production of the marketed drug.

Top down processes [1-4,6,9,10]

One starts from large crystals in the micrometer range and goes down to the nanodimension by diminuting the crystals;such as performing a milling process and using high pressure homogenization [5,11,12]. For the milling method, dry milling (e.g. jet milling) is not efficient to obtain a size in the nanometer range; therefore, wet milling is applied. Wet milling is a means that the drug particles are dispersed in a surfactant/stabilizer solution and the obtained macrosuspension is then subjected to milling energy. The classical milling process is the pearl mill (bead mill), being the NanoCrystal™ technology. Milling media, dispersion medium (generally water), stabilizer and drug are filled into the milling chamber. Shear force of impact, generated by the movement of milling media, leads to the

particle size reduction. The pearls or balls used as milling media consist of ceramics,stainless steel, glass or highly crosslinked polystyrene resin-coated beads [9,10,13]. This technology is an important particle size reduction technology which has been used to produce four FDA-approved drugs such as Rapamune®,Emend®, Tricor®, and Megace ES® [14]. The common problem of this technology is an erosion of milling material during the milling process. To solve this problem, coated milling beads are used to reduce the impurities caused by erosion of milling media. Another problem is an adherence of product to the inner surface area of the mill (consisting mainly of the surface of the milling pearls and the surface of the mill itself).For the homogenization method, there are three important technologies to produce nanocrystals which are Microfluidizer technology (IDD-P™ technology), Piston-gap homogenization in water (Dissocubes® technology) and Piston-gap homogenization in water mixtures or in nonaqueousmedia (Nanopure® technology). The microfluidizer technology can generate small particles by a frontal collision of two fluid streams under pressure up to 1700 bar. This leads to particle collision, shear forces and also cavitation forces. This method can be achieved with jet stream homogenizer such as the microfluidizer. Unfortunately, for the sufficient particle size reduction, it is required a relatively high number of cycles (50-100 passes).The Dissocubes® technology employs piston-gap homogenizers that can produce the nanoparticle suspensions in water at room temperature. A drug powder is dispersed in an aqueous surfactant solution and subsequently forced by a piston through the tiny homogenization gap with pressure up to 4000 bar, typically 1500-2000 bar. The resulting high streaming velocity of the suspension causes an increase in the dynamic pressure which is compensated by a reduction in the static pressure below the vapor pressure of the aqueous phase (according to Bernoulli's law). The simplified form of Bernoulli's law is shown below.

$$p + q = p_0$$

where p_0 is total pressure, p is static pressure, q is dynamic pressure. Formation of gas bubbles occurs because the water starts boiling at room temperature. The gas bubbles collapse immediately when the liquid leaves the homogenization gap being again under normal air pressure of 1 bar. The phenomenon of formation and implosion of the gas bubbles is called cavitation resulting in shockwaves. The drug particles are reduced in size due to high shear forces, turbulent flow and the enormous power of these shockwaves. However, the use of water leads to many disadvantages such as hydrolysis of water-sensitive drugs and problem during subsequent drying steps. Another approach using the piston-gap homogenizer is the Nanopure® technology. The dispersion media with a low vapor pressure (e.g. oils, PEG or hot-melted polyethylene glycols) and optionally homogenization at low temperatures are used in this technology. The cavitation in the homogenization gap is very little or nonexistent. Even without cavitation, the size diminution to achieve nanoparticles is sufficient by the remaining shear forces, particle collisions and turbulences. A low temperature while homogenizing makes this process suitable for temperature labile drug. Also,it is possible to carry out the whole process in nonaqueous media to protect the drug from hydrolysis. The obtained suspensions from Nanopure® technology can directly be filled into soft gelatin capsules or into hard gelatin or HPMC capsules which are then being sealed. In addition, drug nanocrystals in solid PEG can be used as powder for tablet production [15].

To obtain an optimized formulation for the homogenization method, the following process parameters that influence on properties of nanocrystals must be considered such as:

1. Applied pressure
2. Number of homogenization cycles
3. Temperature

Usually, the homogenizer can handle varying pressures,ranging from 100 to 1500 bar for most lab-scale ones. Therefore,an effect of homogenization pressure on the particle size should be investigated to optimize the final formulation. The pressure is provided by the pump converting the kinetic energy of the fluid in the gap. The higher homogenization pressure, the higher velocity of the fluid in the gap is. The static pressure will drop to a larger extent leading to generating more bubbles and then higher energy to comminute the particles. This is consistent with the law of conservation of energy. Therefore, it is anticipated that the higher homogenization pressure, the smaller particle sizes are obtained.

Usually for the production of the drug nanocrystals, a maximum pressure (for most lab homogenizers this value is 1500 bar) is required. The fluid passing through the gap is performed instantaneously, generally within several milliseconds. The energy generated in such short time is not

sufficient to comminute all particles into uniform drug nanocrystals even at the highest applied pressure 1500 bar; thus more homogenization cycles are needed to perform. The increased cycle numbers provide more energy to break down the crystals. Therefore, homogenization is often performed in five, ten, or more cycles depending on the hardness of drug and the desired particle size. Apart from reducing the particle size, more cycles lead to more homogenous nanocrystal suspensions, i.e. a narrow size distribution. Because the flow rate of fluid in the gap is not identical among different zones and the fluid in central zone of the pipe has the higher velocity than the fluid near the wall, the energy dispersed among the fluid is not uniform, leading to an inhomogeneous particle size distribution. By increasing number of cycles, the probability that larger particles pass the zone of high-power density in the middle of the gap increases; thus these particles are also diminished. Therefore, the particle size is a function of pressure and number of cycles. The desired particle size can be achieved by adjusting the procedure parameters, pressure and cycle number. Temperature is also an important parameter which should be strictly controlled when the drug is temperature sensitive. High pressure processing increases the temperature of the sample (approximately 10º C at 500 bar). An increasing temperature in the homogenization process is not favorable to temperature-sensitive drugs. In that case, the temperature can be promptly reduced by placing a heat exchanger ahead of the homogenizer valve. In general, the sample temperature can be maintained at about 10ºC and even below so that the process is applicable to the temperature-sensitive drugs. High pressure homogenization is a simple technique.When an optimized procedure is achieved after adjustment of the production parameters, high quality nanosuspensions with little batch-to-batch variation can be obtained. An important advantage is that a considerably high productivity can be obtained with very low microparticle content in the product. In addition, compared with pearl milling technique,the contamination due to the erosion from the wall of the homogenizer is at a lower level. Muller et al. investigated the metal contamination of the nanosuspensions under a harsh production condition, i.e. 20 cycles at a maximum pressure of 1500 bar. The most dominant iron ion in steel was analyzed in nanosuspensions and was found to be below 1 ppm, which was an uncritical level and safe even for a chronic therapy.

Other techniques for the production of drug nanocrystals [2,4,6,9,10]

Milling, high pressure homogenization, and precipitation are main methods employed for the production of drug nanocrystals. However, there is an intensive research for newtechnologies leading to many other approaches for the production of drug nanocrystals. The combination technologies combine generally a pre-treatment step followed by a high energy process, such as the NanoEdge™ technology. In the first step, crystals are precipitated; and the obtained suspension is then subjected to a high energy process, typically used high pressure homogenization. Smart Crystal technology is not only one technology but a number of different processes that are combined either to accelerate production by reducing the number of passes through the homogenizer or to obtain very small nanocrystals below 100 nm. Such small nanocrystals are difficult to produce via pearl milling or simple high pressure homogenization, especially in large scale industrial production. The combination process H69 is a parallel flow precipitation and subsequent high pressure homogenization (HPH) in which the precipitation takes place in the cavitation zone or just before the cavitation zone of the homogenizer (cavi-precipitation). In the H42 process, spray-drying and high pressure homogenization are combined. Moreover, in H96 process, the most effective combination technology, the lyophilization (bottom up) and the high pressure homogenization (top down) are combined to yield nanocrystals of the size significantly smaller than 100 nm. Recently, a novel tricombination technology as "Precipitation-lyophilization-homogenization (PLH) method" for preparation of nanocrystals had been proposed by Janyaprasert group [16].

This combination technology composed of precipitation, lyophilization and homogenization techniques, respectively. First step was the precipitation process which was used to reduce an initial particle size of the drug. In this step, the drug was dissolved in an organic solvent and added into an aqueous phase, resulting in a precipitation of preferably friable and small crystals. The organic solvent was carefully removed from the nanosuspensions to avoid its cosolvent action which may result in particle growth. Afterward, the second lyophilization step was applied which led to modification of the starting material and removal of the organic solvent used in the precipitation step. Finally, in the last step, high pressure homogenization was applied to break the crumbly particles into the nanometer range. Diagram of preparation step of PLH technique is shown in Fig. 1. The results showed that PLH technique could provide an effective reduction of particle size of clarithromycin nanocrystals to approximately of 400 nm with homogeneity size distribution after only the fifth cycle of homogenization whereas the

same size was attained after 30 cycles by the normal high pressure homogenization (HPH) technique [27].Among other technologies, the following supercritical fluid methods are also mentioned to produce nanocrystals such as rapid expansion of supercritical solution (RESS), rapid expansion from supercritical to aqueous solution (RESAS),solution-enhanced dispersion by the supercritical fluids (SEDS), spray freezing into liquid (SFL), evaporative precipitation into aqueous solution (EPAS), and aerosol solvent extraction (ASES).In vivo performances of drug nanocrystals in oral administration routes In contrast to other nanoparticle systems, drug nanocrystals consist mainly of pure active drugs. Drug nanocrystals exhibit many advantages including high efficiency of drug loading, easy scale-up for manufacture, relatively low cost for preparation and applicability to various administration routes, such as oral [17,18], parenteral [19,20], ocular [21-23], pulmonary [24-26]and dermal [27-30]delivery. As known, the oral route is the most important and the first choice for drug delivery because of its several advantages including convenience, safety, inexpensive, etc. Poorly water soluble drugs for oral administration often show many problems in bioavailability including, a low/variable bioavailability, a retarded onset of action, a variation in bioavailability resulting from fed/fast state and a large oral dose usage. The production of drug nanocrystals offers many advantages for oral drug delivery and provides a solution to these problems.Additionally, at present, the formulations of drug nanocrystal in the market are mostly used for oral delivery.

Drug nanocrystals could improve an absorption of drug due to two major mechanisms via firstly, an improvement of solubility and dissolution rate and secondly, the bioadhesion to the intestinal wall. For the firstly aspect, drug absorption in oral administration is involved with the process that drug is dissolved from the formulation into aqueous digestive fluid and then it is transported across the GI epithelium into the blood circulation. The dissolution is generally considered to be the rate-limiting process in oral delivery of the drugs in BSC class II. Drug with poor solubility and dissolution rate will provide a slow and erratic dissolution that limits the in vivo absorption and is unable to reach an effective therapeutic concentration. The formulation of drug nanocrystals can impressively improve the bioavailability of per orally administered poorly soluble drugs as shown by changes in pharmacokinetic parameters of blood profiles including, an increase in area under the blood concentration time curve (AUC), an increase in maximum plasma concentration (Cmax),a decrease in time to maximum plasma concentration (Tmax).For example, Liversidge and Cundy reported that danazol, a gonadotropin inhibitor, showed the absolute bioavailability of marketed danazol microsuspension (200 mg, 10 mm) only 5.1 ± 1.9%.

Meanwhile, the absolute bioavailability of danazol nanosuspension (200 mg, 169 nm) was 82.3 ± 10.1% which was equal to 16-fold increase in bioavailability. Additionally, the Tmax was reduced and the Cmax was 15-fold increased [17].Amphotericin B was formulated as a nanosuspension for the treatment of visceral leishmaniasis. After oral administration (5 mg kg−1) in BALB/c mice, amphotericin nanosuspension could significant reduce liver parasite numbers in the liver by 28.6% compared to untreated controls. While, the micronized amphotericin B did not show any curative effect [31]. Additionally, the formulation of drug nanocrystals can provide advantage whenever a quick onset of a poorly soluble drug is required. For instance, an analgesic naproxen was formulated as nanosuspension (270 nm) for oral administration. Besides the nanosuspension of naproxen was approximately 3-fold increased in AUC when compared to an unmilling suspension (20 mm), it could be concurrently reduced in Tmax. The data showed that the time for nanosuspension to reach Cmax was only about 8 min whereas the unmilling naproxen suspension was achieved the Cmax at 33.5 min. It was suggested that an increase in 4-fold faster absorption rate of nanosuspension when compared to unmilling suspension was contributed to the increased solubility and dissolution rate of nanocrystals [32]. In the study by Li et al., revaprazan hydrochloride was developed in form of nanosuspensions. The in vivo evaluation showed that revaprazan hydrochloride nanosuspensions exhibited significant increase in AUC0-t (45%and 36% higher), Cmax (87% and 98% higher) and decrease in Tmax (185 and 315 min shorter), MRT (114 and 157 min shorter) when compared to a coarse suspension [33]. Nitrendipine nanosuspensions were prepared by precipitation ultrasonication method to enhance the dissolution rate and oral bioavailability of the drug. The in vivo test demonstrated that the Cmax and AUC $_{0\text{-}12}$ values of nanosuspensions in rats were approximately 6.1-fold and 5.0-fold greater than that of commercial tablets, respectively [34].

These examples obviously demonstrated that nanocrystals formulation could increase dissolution velocity and saturation solubility of poorly soluble drugs. Therefore, the fast and complete drug dissolution,an important prerequisite for drug absorption, is achieved.The second mechanism of nanocrystals that can improve the drug absorption is due to the mucoadhesion to biological mucosa (GI

mucosa) which can positively influence the oral bioavailability. Owing to the adhesiveness of nanocrystals to GI mucosa, drugs can provide the higher concentration gradient and prolonging residence and contact time in the GIT. The mucoadhesion mechanism of nanoparticles could be explained by many theories including, the electronic theory (electrostatic attraction forces between the surfaces of particles and mucus), the adsorption theory (hydrogen and van der Waals bond between the surfaces of particles and mucus), the diffusion theory (interpenetration and physical entanglement of the mucus protein and polymer chains), and the trapping theory (retention of nanoparticles by the uneven mucosa surface). Due to the benefits of mucoadhesion, some researchers were interested in an enhancement of adhesiveness between nanocrystals and GI mucosa by modifying the surface of drug nanocrystals with cationic polymers or incorporation of drug nanocrystals into mucoadhesive polymers. Additionally, the utilized mucoadhesive polymers can prevent the drug from degradation. The antibiotic buparvaquone, used for treatment of Cryptosporidium parvum (C. parvum), has very low oral bioavailability due to its low solubility. Nanosuspension of buparvaquone cannot only increase drug solubility but it can also perform a mucoadhesion to the gut wall. In addition, an incorporation of buparvaquone nanosuspension into mucoadhesive polymers can enhance the mucoadhesive and show more effectively clear C. parvum from the GIT when compared to the unmodified nanosuspension [35].

Another problem of poorly soluble drug is a variation in bioavailability resulting from fed/fast state. Poorly soluble drugs usually shows an increased or accelerated absorption when intake with food. Drug bioavailability is increased due to the food effect because of the enhanced dissolution rate in GIT caused by several factors including larger volume of the gastric fluid, delayed gastric emptying, increased bile secretion,increased gastric pH (for acidic drugs), and increased splanchnic blood flow [36]. When poorly soluble drugs are formulated as a uniform nanosuspension, the variation in bioavailability resulting from fasted/fed state can be minimized. The nanocrystals could significantly increase dissolution rate because of the increase in solubility and enormous particle surface. The dissolution rate of nanocrystals is fast enough even under the fasted state. Therefore, the absorption in both fasted and fed state can be a permeability-limit, and the absorption difference between the fasted and fed conditions due to the dissolution difference is eliminated. For example, the formulation of cilostazol nanocrystals (220 nm) could significantly reduce fed-fasted ratios of the Cmax, AUC, Tmax and MRT as compared to a microsized dispersion (13 and 2.4 mm) when given in beagle dogs. Therefore, the fasted/fed variation in bioavailability was almost eliminated [36]. The study of Wu et al. showed that the nanocrystals dispersion of aprepitant (MK-0869), the active ingredient in Emend®, could eliminate the food effect on oral absorption. The fed-fasted ratio was reduced and the bioavailability was improved in the beagle dogs at a dose of 2 mg/kg [37]. The same result was also found in the study by Sauron et al. The food effect on bioavailability of a new tablet formulation containing fenofibrate nanoparticles was accessed in human. It was demonstrated that the peak and overall exposures from the 145 mg nanoparticle fenofibrate tablet were not affected by food and the result was concluded that the nanoparticle fenofibrate tablet can be taken regardless of the timing of meals [48].

Poorly soluble drugs usually provide more troublesome in the safety issue because of the use of a large amount of organic cosolvent or solubilizer that will result in an unwanted side effect or toxicity. Drug nanocrystals are generally reported as a safe and well tolerated formulation in many administration route compared with the conventional products.Several safety advantages of drug nanocrystals in oral delivery include i) fine particle size, ii) safe composition, and iii) tolerance to various sterilizations. The fine particle size of drug nanocrystals can increase the distribution uniformity in the gastrointestinal fluid and avoid the high and prolonged local concentration [32]. Nanocrystals are also beneficial to a better toleration in the mucosa delivery by reduction in the occurrence of the local irritation or gritty feel. For example, the study by Liversidge and Conzentino demonstrated that naproxen nanosuspensions showed not only the faster onset of action but also a reduction in the gastric irritancy [32]. Drug nanocrystals can provide an opportunity to escalate dose and reduce solvent-related adverse effect because of the safe composition since nanosuspension formulations do not require organic solvent or extreme pH ranges for solubilization of poorly soluble drug [11]. Additional benefit of drug nanocrystals in safety issue is the tolerance to various sterilizations. Several sterilization approaches can be successfully applied to nanosuspensions including gamma radiation, filtration sterilization, and thermal sterilization. Concerning the final formulations of drug nanocrystals, most drug nanocrystals in the in vivo experiments are aqueous dispersions.

In clinical application, liquid dosage forms might be suitable for some groups of patients, e.g. children or elderly patients, but not for normal patients. In general, solid dosage forms are usually more preferred. Therefore, the liquid nanosuspensions should be transformed into dry powders which are then used for production of tablets, capsules, or pellets. There are several methods that can be used for solidification this nanosuspension. In case of drug nanosuspensions in pure water or in water containing mixture,nanosuspensions may be used as a granulation fluid for further production of tablets. The nanosuspension is admixed to binders and other excipients, and the granules are then finely compressed into the tablets [39]. Furthermore, nanosuspensions can also be produced as matrix pellets or layering dispersion in fluidized bed process [40-42]. In case of drug nanosuspensions produced in nonaqueous media such as liquid/solid PEG, the use of melted PEG which is solidified at room temperature for the dispersion of nanosuspension is interesting. After solidification of PEG, the nanocrystals containing mass can be ground and filled into the capsules. Additionally, the other approval methods for solidification of nanosuspension are such as spray-drying and lyophilization.

Spray-drying process is the cost effective approach to transform the nanosuspensions into dry products under appropriate conditions. Lyophilization process is recommended for Intravenous product in order to avoid aggregation or caking of settled drug nanocrystals. However, during the drying process, the particle aggregation should be considered since the benefits of nano-sized particles will be lost if the particle aggregation occurs. Therefore, an addition of protectants (usually sugars) may reduce the growth of particle size during a solidification process. Besides the transformation to dry powder of nanosuspensions, the redispersion of solid drug nanocrystals in gastrointestinal fluid should be concerned.The stabilizers attached to the nanocrystal surfaces that provide efficient ionic or steric repulsion and have no effect from the GIT environment should be used.Regardingmicromeritic aspects, drug nanocrystals provide high saturation solubility and consequently increase dissolution velocity. However, in some applications, drug nanocrystals are essentially combined with traditional controlled release technology (e.g. coated pellets) to avoid excessively high plasma peaks and premature time to reach maximum plasma concentration (t_{max}), and to achieve prolonged blood levels. Besides an optimal drug nanocrystal size and crystalline/amorphous state that are taken into account for the production of drug nanocrystals, the other factors including the required blood profile, administration route, and stability of the amorphous state during shelf life of the product should be in consideration [4]. In addition to oral administration, drug nanocrystals also play a beneficial role on other administration routes. They can create supersaturated systems with high thermodynamic activity for dermal delivery; create systems with prolonged retention times for ophthalmic administration; create mucoadhesive systems for mucosal administration of nasal,vaginal and pulmonary. Furthermore, an administration of drug nanocrystal suspensions as parenteral formulation is also feasible. The surfaced-modified drug nanocrystals can be preferentially adsorbed onto blood proteins for site specific localization that is applied as a targeted drug delivery.

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 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. To increase the dissolution and bioavailability of nanocrystals, it is preferable to formulate the nanocrystals in a metastable crystalline form or even prepare the amorphous equivalent of nanocrystals. However, this is not being practiced commonly. 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. Therefore if the drug is susceptible to hydrate formation, then the potential or factors triggering the said conversion should be extensively investigated during stability studies in different conditions [49]. X-ray powder diffraction

(XRD), thermal analytical techniques (differential scanning calorimetry, thermogravimetry, etc.) and vibrational spectroscopy (infrared and Raman) are the most commonly used methods to determine and monitor the solid state form of nanocrystals.

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. The DSC of pure drug, physical mixture of drugand excipients (stabilizer) and final formulation which may be in dried form is done. DSC can be classified based on the mechanism of operation, into two classes; heat flux DSC and power compensated DSC. In heat flux DSC, two pans are placed on a thermoelectric disk surrounded by a furnace containing sample and empty reference pan. The furnace is heated at a linear heating rate, and the heat is transferred to the sample and reference pan through the thermoelectric disk [49-51]. 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 = DT/R where q is "sample heat flow", T is "temperature difference between sample and reference", and R is "resistance of thermoelectric disk" [50]. In a power-compensated DSC, the sample and reference pans are placed in separate furnaces heated by separate heaters [49,51]. 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. Kocbek et al. prepared nanosuspension of ibuprofen using Poloxamer 188 as a stabilizer. A single exothermic peak at an onset temperature of 74.8 ºC was seen in the DSC curve of pure ibuprofen, due to its melting. The DSC curve of Poloxamer 188 also manifests a single endothermic peak with an onset temperature of 51.4º C. Two distinct endothermic changes in the DSC curve of freeze-dried ibuprofen-Poloxamer 188 nanosuspension were observed. The first endothermic change appears as a tall narrow peak with an onset temperature at 39.4 ºC and the second as low broad peak with temperature of maximum at 56.8ºC, where it was not possible to analyze the onset temperature. These results indicated the formation of a eutectic mixture of the drug and Poloxamer 188. In this case, the peak at the lower temperature represents the melting of the eutectic system, and the second, change at the higher temperature, represents the melting of the excess component. Based on the position of the second peak, it can be estimated that ibuprofen is the surplus component left after the eutectic has been formed. Therefore the increased dissolution rate of the drug can be explained by formation of the eutectic system and submicron sized drug crystalsproduced during the formulation of nanosuspensions [52].

Among other thermal techniques, hot stage microscopy (also known as Thermal Microscopy or Thermomicroscopy) is a combination of microscopy and thermal analysis to enable the study and physical characterization of materials as a function of temperature and time. Hot stage microscopy not only aids in screening and characterization of polymorphs, but also identification of crystalline and amorphous region of nanocrystals. Yin et al. developed nanocrystals of a new drug moiety BMS-347070 by spray-drying with a surfactant Pluronic F127. The authors used hot-stage microscopy to compare the drug processed with Pluronic F127 and micronised pure drug. The hot stage microscopy images of the co-processed and micronized drug were collated at 100x magnification. Both the samples were put on slide in the same field of view. The slide was heated to 250 ºC at a rate of 10 ºC/min. The drug particles remaining in the molten Pluronic were found to be much smaller compared to the pure drug, the size of which cannot be determined by optical microscopy [53].Thermal analysis may also be performed by thermogravimetric measurements or differential thermal analysis (DTA). Huang et al. used DTA for the thermal analysis of SKLB610 nanosuspensions at a heating rate of 10ºC/min in the range of 25–600ºC. The DTA curve of SKLB610 displayed a drug melting peak at 155.7ºC. No such peak was observed with the nanoparticles. Pure SKLB610 was also linked with another peak at 132–133ºC. The melting peak of SKLB610 in nanosuspension was relatively blunt as compared to that of pure SKLB610, apparently due to the preparation process.Amorphous domains were also generated on the particle surface [54].

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. Koneti et al. compared the top-down and bottom-up approaches for the preparation of nanosuspensions of glipizide based on their XRD output. The authors carried out XRD to analyze the modification in the crystalline nature of the drug following its conversion into nanocrystals. The diffraction pattern of pure glipizide powder manifested several sharp high intensity peaks at multiple diffraction angles (2θ values) signifying that the drug existed as a crystalline material.Glipizide nanosuspension displayed peaks almost similar in intensity and position, indicating that the crystallinity of the drug is intact when the nanosuspension is produced by liquid antisolvent precipitation method. 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 [55].

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. Based on the position IR peaks in the formulation compared to that of the pure drug, it was concluded that milling and spray drying did not change the chemical composition of curcumin [56].

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. This phenomenon is referred to as the “Raman Effect”. This shift provides information about vibrational, rotational and other low frequency transitions in molecules.Waard and colleagues developed a novel bottom-up process to fabricate drug nanocrystals termed as “controlled crystallization during freeze drying” (CCDF), wherein a solution of an organic solution of a poorly water-soluble drug and an aqueous solution of a matrix material are mixed and freeze dried at specific conditions to allow the drug and the matrix to crystallize. 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. The sample to be studied comprised of the drug (Fenofibrate; FNB), the solvent (tert-butyl alcohol; TBA), mannitol and water. The Raman probe was placed directly above the sample in the freeze dryer and individual stages of the CCDF process were separated and prolonged to allow measurement of the complete phase changes. CCDF comprises of three consecutive steps: freezing, increasing the temperature, and drying. The in-line Raman measurements showed that the first two steps, the freezing step and the crystallization step are critical steps that determine the final size of the fenofibrate crystals [57].

As mentioned earlier, the solid-state characteristics of nanocrystals are influenced by the production method. With bottom-up techniques partial amorphousness is commonly manifested,with pernicious effects on the stability of the nanocrystals. 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. However, if the process is performed in an aqueous environment, water serves as a plasticizer (raising molecular mobility) and reduces the propensity for sustained formation of amorphous material. Ali et al. prepared hydrocortisone nanosuspensions by both wet-milling and microfluidic nanoprecipitation [58]. With both methods, the particle sizes were approximately 300 nm,

yet the product obtained via milling was crystalline, while precipitation resulted in a predominantly amorphous product. In vivo tests with rabbits following ocular delivery, demonstrated comparable bioavailability with both the formulations and when compared against the drug solution, the bioavailability was found to be almost two-fold. However, the differences were evident in stability tests, the crystalline wet-milled nanosuspension was stable for two months (unrevised particle size), but the particle size of the amorphous precipitated nanosuspension had increased to 440 nm. Lai et al. formulated piroxicam nanocrystals with poloxamer 188 as a stabilizer by high pressure homogenization [**59**]. While the raw material was predominantly form I, a mixture of monohydrate and form III composed the formulated nanocrystals. The solubility of form I is 14.3 mg/L, while that of form III is 17.0 mg/L. In this case, the solubility was increased not only due to the smaller particle size, but also due to the formation of the higher energy solid-state forms. Pireddu et al. studied two different diclofenac sodium crystal forms for transdermal drug delivery [60].

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. In vitro penetration and permeation studies revealed that all the nanosuspension formulations demonstrated an improved drug penetration compared to the commercial gel formulation. Interestingly, though the two polymorphic forms varied in their permeation properties; when administered as coarse suspensions, their nanosuspensions behaved similarly.

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 mean particle size of nanosuspensions is typically analyzed by dynamic light scattering also known as photon correlation spectroscopy (PCS) [61]. It has advantages of yielding accurate results and fast and easy measurement. However, this technique is not feasible to analyze particles larger than 6 μ. Apart from the mean particle diameter, PCS can also yield the width of the particle size distribution, referred to as the "polydispersity index" (PI). The 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 advantage of light microscopy is the visible and therefore yields a doubt free result, however, a major drawback is lack of any statistical significance because it is not possible or very time consuming to analyze 10,000 particles or more, necessary for a valid analysis. Laser diffractometry (LD) is a robust technique and has the advantage over all the other techniques to be able to analyze large particles, small nanoparticles and mixtures of small and large particles within only one single measurement. The 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). However, practically it is only possible to analyze particles from about 400 nm and larger using this technique [61].

Laser diffraction is not feasible for measurement of particles smaller than about 400 nm, because the intensity of diffracted light decreases with decreasing size. However, modern LD instruments can analyze particles starting from 20 nm up to those in micron range or even larger. The

appendage of the measuring range for very small particles was possible by introducing a second, supplementary technique, into the measurement. The additional technique gains more information about the particles by determining other optical phenomena (e.g., scattering intensities in different directions) [62,63].Thus, the additional techniques are different to pure laser light diffraction. The additional information from the supplementary technique is then amalgamated into the size analysis of the LD measurement, which leads to a pooled result of pure LD and supplementary technique. Therefore, strictly spoken,today's LD measurements are not only pure LD measurements, but an integrated report of two different techniques. However, the additional technique may also overestimate the presence of the nanoparticles by overlooking larger particles (e.g., large crystals and/or aggregates/agglomerates) [64]. This finding is of extraordinary importance, because most often LD is used to detect possible large particles besides a nanosized major bulk population, or to evidence the absence of such large crystals, which is not possible in PCS measurements. Hence to conclude, particle sizing of nanocrystal formulations will only lead to meaningful results if all the aforementioned parameters are considered. Moreover, it should be noted that the particle size data of a nanosuspension obtained by LD and PCS are dissimilar, because the LD data are volume based and the PCS mean particle size is a light-intensity weighted size.

A Coulter counter analysis is essential for nanosuspensions to be administrated intravenously.In contrast to the volume distribution of the LD analysis, the Coulter counter data give an absolute value that is the absolute number of particles per volume unit for the different size classes. The size of the smallest blood capillary is about 5µm, so even a small content of particles greater than 5µm may cause capillary blockade or emboli formation. Hence the content of microparticles in nanosuspensions should be controlled strictly by Coulter counter analysis [65].

Obstacles in particle size analysis not only arise from the instrumental setup alone, but also from the material to be analyzed. General concept states that the stability of the sample during analysis is the most important prerequisite for correct and reproducible results [66]. However this is not always easy to achieve and sometimes changes are not even deciphered. Possible alterations or instabilities of a sample are for instance agglomeration or dissolution. Therefore the analysis of samples with high solubility and/or increased dissolution velocity might be especially sensitive to such changes. Apart from these, the other techniques used for the particle size analysis include confocal laser scanning microscopy, scanning probe microscopy, and scanning tunnelling microscopy.

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.Principally, agglomeration may be observed following water removal, leading to an increase in the particles size. Such changes and more can be observed through SEM. To play down the magnitude of the increase of particle size, some excipients are included as "protectants". Mannitol is usually used as a cryoprotectant in lyophilization, which recrystallizes around the nanocrystals during the water-removal operation, thus preventing particle interaction and agglomeration. Agglomeration within a certain limit is allowable when the final particle size is still in an acceptable range. Moreover,the dried powder should be easily redispersed into stable nanosuspensions. The shape of the drug crystals depends on their crystalline structure [65,66].

Atomic Force Microscopy (AFM)

It is a kind of scanning probe microscope is designed to measure local properties, such as height, friction, magnetism with a probe. AFM was used to investigate the morphology and surface properties of probucol nanocrystals following dispersion of probucol/polyvinylpyrrolidone (PVP)/sodium dodecyl sulfate (SDS) ternary ground mixture into water. The observed particles had core-shell structure, i.e., drug nanocrystals enveloped in aPVP and SDS complex. The AFM phase image and the force curve analyses indicated that probucol nanoparticles with PVP K17 manifested layer structure, compared to those with PVPK12. The structural difference was accountable in terms of the molecular states of PVP-

SDS complex on the particle surface.These findings support not only the mechanism of drug nanoparticle formation but also the in vivo absorption results with the almost same particle size of 40 nm [67].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. Both techniques displayed a similar level of efficiency of binding to the solid surfaces [68]. The interaction measurements were corelated to successful formulation of nanocrystals with the same drug-stabilizer systems by wet ball milling. Hence, it was concluded that merely the interaction forces cannot determine the most efficient stabilizer, but moderate affinity with longer PEO chains, which are efficient for steric stabilization,formed best nanosuspensions.

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. Aerodynamic diameter is a critical parameter that determines the lung deposition of the DPIs. Different particle shapes yield different drag forces and particle terminal settling velocities, which in turn influence the lung deposition of the DPIs. Enhancing particle surface roughness would ascribe to decreased aerodynamic diameter of the particles. This would inturn have a higher possibility of deep lung deposition as compared to spherical particles. In another study, elongated particles were found to have better aerodynamic behavior as compared to spherical ones. Particle aerosolization is another factor responsible for deep lung delivery.Aerosolization is mainly influenced by the interaction between the particles and between the particle and the wall of the inhaler. Particle interactions are linked to the van derWaals forces, which are the particle surface morphology, size, shape, electrostatic properties and hygroscopicity. Particle shape that possess low contact area and van derWaals 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. A study by Hassan et al. evaluated the flowability, aerosolization, and deposition properties of particles of different shapes like sphere, cube, needle, and pollen [69]. Pollen shaped particles are found to display better flowability, aerosolization, and deposition properties compared with other particle shapes.

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.In general particles inherently possess a surface charge, owing to the dissociation of surface functional groups, referred to as the Nernst potential. The degree of dissociation of the functional groups depends of the pH of the suspension, therefore zeta potential is a pH dependent characteristic.

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 second monolayer absorbed consists of positively charged, fixed but hydrated ions, referred to as the outer Helmholtz layer. Both Helmholtz layers together are designated as the Stern layer. The uncompensated negative charge of the surface is compensated by freely diffusing counter ions in the so called "diffuse layer". The border of the diffuse layer is defined where the particle surface charge is fully compensated. The zeta potential is determined by measuring the electrophoretic particle velocity in an electrical field. During the particle movement the diffuse layer is shed off, hence the particle acquires a charge due to the loss of the counter ions in the diffuse layer.This charge at the border of the shedding is termed as the zeta potential.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 generaly 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 ºC,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.The measurement of the zeta

potential allows the prediction about the storage stability of submicron colloidal dispersion [66,70]. In general, particle aggregation is less likely to occur if particles possess enough zeta potential providing sufficient electric repulsion, or enough steric barrier providing sufficient steric repulsion between each other. According to the literature, a zeta potential of at least -30 mV for electrostatic and -20 mV for sterically stabilized systems is desired to obtain a physically stable nanocrystal suspensions . The upper limit for the zeta potential is +30 mV.

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. Since apparent solubility of nanosized particles is higher than the thermodynamic solubility of the material, dissolution of nanocrystalline material is likely to lead to a supersaturated solution. This is termed as the "spring effect". Ige et al. studied the saturation solubility of fenofibrate nanocrystals, which had been reduced in size from 80 μm(bulk drug) to 460 nm (nanocrystals). 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 107μg/mL, respectively [71].

In another study, the intrinsic dissolution rates and surface concentrations of indomethacin nanocrystals with two different poloxamer stabilizers were studied [72]. Intrinsic dissolution rates were measured with a channel flow system. 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 _g/min/mm2, while that for poloxamer F127 was 0.31 μg/min/mm2. The dissolution rate of bulk indomethacin was also determined, and found to be considerably lower at 0.05 μg/min/mm2. Surface concentrations have also been measured with UV-imaging. Herein again, differences in concentrations were affected by both particle size and stabilizer. For e.g., for nanocrystals of a uniform size (580 nm), the surface concentration after 10 min of dissolution was 28.7 mg/L for Pluronic F68 while with Pluronic F127 the concentration was 22.1 mg/L.The surface concentration with bulk indomethacin was only 2.1 mg/L.Liu with coworkers studied the effect of ultracentrifugation and filtration on the dissolution results.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 wavelength for the analysis. Sarnes with colleagues determined drug concentrations in solubility testing of nanocrystalline samples with UV-spectrophotometer. The drug concentration determinations were performed at wavelength equivalent to the absorption maximum of the drugs; while the absence of undissolved particles was confirmed with the wavelength at which the absorbance of the excipient and drug was negligible [68].

Eventually, precipitation may sooner or later occur until the concentration is equivalent to the thermodynamic solubility. Furthermore, changes in the composition and pH of the solution such as in the gastrointestinal tract will affect solubility and hence thetendency for crystallization.Therefore, the supersaturated state should be maintained and precipitation hindered to enhance in vivo bioavailability. Some polymers, such as polyvinyl pyrrolidone (PVP), methacrylate co-polymers, hydroxypropyl methylcellulose (HPMC), and hydroxypropyl methylcellulose acetate succinate (HPMC-AS) are effective at maintaining (or at least helping to maintain) supersaturation. This is known as the "Parachute effect". Solid dispersions, especially where the amorphous drug is dispersed on a molecular level within the polymeric crystallization inhibitor, are well established as parachute promoters. However, the permeation from supersaturated solutions may be thwarted by the precipitation inhibitor, as is the case often with solubilizing agents, when the drug favors the formation of micelles instead of permeation [72,73].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. Second, polymers adsorbed on solid surfaces (e.g., with nanocrystals) can block the interaction of already dissolved drug molecules with crystal surfaces and thereby crystal growth. Electrostatic bonds, van derWaals' forces or hydrogen bonding can all influence the interaction between the polymer and crystal faces, and therefore the degree of crystal growth inhibition. Moreover, the viscosity of the polymer solution may also inhibit the diffusion of the molecules, which limits crystal growth [74].

Ghosh et al. formulated nanocrystals from a poorly soluble drug with TPGS or TPGS with a co-stabilizer (HPMC, PVP, poloxamers). In vivo studies with dogs revealed a 9 times higher AUC value and 5 times higher Cmax values for the nanosuspension against the coarse drug formulation.The physical stability during storage with TPGS alone was considerably lower than for the mixed systems [75].Ueda et al. studied the maintenance of supersaturation with amorphous and nanocrystalline formulations of carbamazepine. The phenomenon of supersaturation was further studied by conducting real-time monitoring with NMR spectroscopy of the dissolved drug with both amorphous and nanocrystalline drug in supersaturated solution. Based on 1H-NMR measurements, the dissolved concentrations for nanocrystalline carbamazepine were nearly constant for 50 h. Based on this, the authors concluded that nanocrystal formation lowered the degree of supersaturation, leading to a relatively stable supersaturated solution of carbamazepine. The presence of nanoparticles also suppressed the formation of large precipitates. For spray dried amorphous carbamazepine, the initial concentration was higher but it then dropped below the concentration of the nanocrystalline sample, demonstrating that the higher supersaturation was more kinetically unstable with fast precipitation/crystallization of large microparticles. The particle size of nanocrystals in this study was approximately 150 nm [76].

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 [77,78]. 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. The factors including the particle size of the drug crystals, surface properties of the carrier,drug-stabilizer interaction are to be considered while poorly soluble drug is formulated for dermal drug delivery. 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. Nonionic surfactants are preferred over ionic because they are generally less irritating [79]. The permeation studies are usually done by using the Franz diffusion cell apparatus. The human cadaver skin, pig ear skin, rat skin, and pig skin can be used for the same. The nanosuspension is compared with the coarse suspension or the marketed one. The dermal absorption of formulation in vitro is done by application of the test substance in a suitable formulation to the surface of a skin sample, which is placed between the donor compartment and the receptor compartment of a diffusion cell. Two types of diffusion cells are available i.e., static or flow-through [80,81]. In Static diffusion cells, fresh perfusate is added after each sampling. Flow through cells use a pump to pass perfusate through the receptor chamber and collect flux by frequently collecting the perfusate. Static diffusion cells can be sub divided as horizontal or vertical on the basis of skin orientation. The majority of skin absorption studies are conducted using horizontal cells, with the skin surface open to the air. The inner material of Diffusion cells is inert non-adsorbing with receptor chamber volumes of about 0.5–10 mL and surface areas of exposed membranes of about 0.2–2 cm2 [82,83]. The test should be carried out with minimum of six skin samples. The receptor fluid, which must have an adequate ability to solubilize the test substance, is maintained in contact with under side of the skin from the time of application until the end of the collection of the receptor fluid. A very critical parameter is controlling the temperature of

the receptor fluid throughout the experiment. The skin surface temperature in the diffusion cell should be kept to 32 ± 1 ºC. The receptor fluid in static cells should be well stirred during the study.

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.Li with colleagues studied the effect of drug physicochemical properties on oral bioavailability [84].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 was 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.

Many stabilizers used for nanocrystal products (vitamin E TPGS, poloxamers, polysorbates) are also P-gp inhibitors [85]. PEG chain length (between 200 and 6000 Da) in TPGS may influence the inhibitory activity and the best inhibition is observed with PEG chain lengths of 1100–1500 Da. In some cases, nanocrystals can be taken up by cells. This may be desirable (e.g., with cancer cell targeting) or undesirable (unpredictable pharmacokinetic profiles) depending upon the intended purpose of the nanocrystals. The uptake may vary between cell types and their phagocytotic/endocytotic potential, and the properties of nanocrystals such as size, morphology, stabilizer type, and surface charge.The wide array of applications of nanocrystals, and their potential importance highlights the need, in some cases, for a thorough comprehension of the nanocrystal behavior on the cellular and tissue levels.

Chen and Li [86] studied the mechanism of cellular uptake of paclitaxel nanocrystals. They found out that nanocrystals were internalized by KB cells at higher concentrations compared to the solubilized formulations. Based on temperature dependent internalization and confocal imaging, they concluded that drug nanocrystals were possible to be taken up as solid particles probably via endocytosis. However, the uptake was affected by the surface layer of the nanocrystals. Thus, nanocrystalline chemotherapeutic formulation can perhaps intracellularly form a lethal microenvironment for the cell when the drug nanocrystals are slowly dissolved inside the cells. This can be difficult to access for the solubilized drug. The mean particle size of nanocrystals in this study was from 250 ± 30 nm.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 [87]. 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. However, therapeutic efficacy is limited by overexpressing MDR related proteins like P-gp in resistant tumors. Though nanosized materials can be taken up via endocytosis by cells, but following dissolution into cellular cytoplasm they can be pumped out by P-gp efflux system. Hence, utilization of simultaneous lowering of P-gp activity with endocytosis of nanoparticles can increase the therapeutic efficiency, like was the case with TPGS coated paclitaxel nanocrystals. Drug and P-gp inhibitor should be at the same time inside the cells. This was not realized when TPGS was given in solution together with free paclitaxel molecules, but with TPGS coated paclitaxel nanocrystals it worked. While electron microscopy and fluorescence imaging are the two principle techniques to image the physical interaction of nanoparticles with cells, including their uptake and localization within the cells they have some drawbacks (e.g., lack of chemical specificity and inability to analyze live cells with electron microscopy), and complications with fluorescent labels, including label leaching and overestimation of nanocrystal internalization when the fluorescent labels but not necessarily the nanocrystals themselves enter the cells. Thus, it is worth considering novel analytical techniques in this context.

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) [88].In a proof of concept study that also had clinical relevance, Darville et al. imaged the fate of nonfluorescent nano/micro crystals paliperidone palmitate, an antipsychotic prodrug in macrophage cell cultures and histological sections using CARS microscopy [89]. The commercially available product Xeplionr is a long-acting aqueous suspension for intramuscular injection with a measured median volume based equivalent sphere diameter of approximately 1000 nm.The palmitate prodrug aids to reduce solubility and associated dissolution rate, thereby sustaining the release of paliperidone [90]. In vivo complex and variable pharmacokinetic profiles have been observed and in rats the formation of granulomatous tissue in the region of the intramuscular nanocrystals has been observed. The inflammatory response leads to particle agglomeration, phagocytosis and radial angiogenesis in the rats resulting in multiphasic systemic absorption of the paliperidone. CARS microscopy was used to investigate the fate of the paliperidone palmitate nanocrystals with macrophage cells in vitro and histological sections in situ. 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.

CONCLUSION

The conclusion of the document states that the formulation of nanocrystals significantly improves the drug's solubility and dissolution rate, which potentially enhances its oral bioavailability. The study highlights that the nanocrystal formulation is a promising approach to overcome the challenges associated with the poor water solubility of atorvastatin, making it more effective for therapeutic use.

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TABLE NO:1

Drug	**Company**	**Indication**	**Technology used**	**Dosage form**	**Status**
Sirolimus	Rapamune/ Wyeth	Immunosuppressant	Top-down, Media milling	Tablet	Marketed
Aprepitant	Emend/ Merck	Antiemetic	Top-down, Media milling	Capsule	Marketed
Fenofibrate	Tricor/ Abbott	Hypercholesterolemia	Top-down, Media milling	Tablet	Marketed
Fenofibrate	Triglide	Hypercholesterolemia	Top-down, HPH	Tablet	Marketed
Megastrol acetate	Megace/Par Pharmaceutical	Appetite stimulant	Top- down, media milling	Oral suspension	Marketed
Grieseofulvin	Gris-PEG/ Novartis	Antifungal	Bottom up, coprecipitation	Tablet	Marketed
Nabilone	Cesamet/ Lilly	Antiemetic	Bottom up, coprecipitation	Capsule	Marketed
Danazol	-	Estrogen antagonist	Top-down, media milling	Nano suspension	In- vivo (dog)
Naproxen	-	Anti-inflammatory	Top-down, media milling	Nano suspension	In- vivo (rat)
Cilostazol	-	Anti-platelet agent	Top-down, media milling	Nano suspension	In- vivo (dog)

Ketoprofen	-	Anti-inflammatory	Top-down, media milling	Nanocrystals powder	In- vivo (dog)
Cyclosporine	-	Immunosuppressant	Top-down, HPH	Nano suspension	In- vivo (pig)
Spironolactone	-	Diuretic	Top-down, HPH	Nano suspension	In- vivo (rat)
Itracanazole	-	Antifungal	Bottom up, precipitation	Nano suspension	In- vivo (rat)

Table1: Overview of Drug Nanocrystals for Oral administration in current Marketed during pharmaceutical researches

FIGURE NO:1Diagram of the preparation step of drug nanocrystals by precipitation – lyophilization– homogenization(PLH) technique