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Nanosizing techniques for improving bioavailability of drugs

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Abstract

The poor solubility of significant number of Active Pharmaceutical Ingredients (APIs) has become a major challenge in the drug development process. Drugs with poor solubility are difficult to formulate by conventional methods and often show poor bioavailability. In the last decade, attention has been focused on developing nanocrystals for poorly water soluble drugs using nanosizng techniques. Nanosizing is a pharmaceutical process that changes the size of a drug to the sub-micron range in an attempt to increase its surface area and consequently its dissolution rate and bioavailability. The effectiveness of nanocrystal drugs is evidenced by the fact that six FDA approved nanocrystal drugs are already on the market. The bioavailabilities of these preparations have been significantly improved compared to their conventional dosage forms. There are two main approaches for preparation of drug nanocrystals; these are the top-down and bottom-up techniques. Top-down techniques have been successfully used in both lab scale and commercial scale manufacture. Bottom-up approaches have not yet been used at a commercial level, however, these techniques have been found to produce narrow sized distribution nanocrystals using simple methods. Bottom-up techniques have been also used in combination with top-down processes to produce drug nanoparticles. The main aim of this review article is to discuss the various methods for nanosizing drugs to improve their bioavailabilities.

Keywords

Nanosizing, top-down techniques, bottom-up techniques, combination techniques, wet ball milling, high pressure homogenization and precipitation techniques.

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1. Introduction

Advancements in combinatorial chemistry, high throughput screening, biology and genomics have led to an increase in the number of molecules which can be potential drug candidates. However, poor aqueous solubility and bioavailability has led to failure of more than 40 % of these molecules during the drug development stages (1-4). Regardless of excellent pharmacological activity, many of these Active Pharmaceutical Ingredients (APIs) fall under Class II of the Biopharmaceutics Classification System (BCS). The BCS system classifies drugs into four categories depending on their aqueous solubility and membrane permeability (5). To overcome problems like poor aqueous solubility, the pharmaceutical industry and researchers have focused on developing formulation strategies for drugs classified under BCS class II (poor solubility and high permeability) and BCS class IV (poor solubility and permeability) (2, 5). A large number of approaches have been developed to improve oral bioavailability of these compounds by increasing their aqueous solubility and dissolution rates.

Conventional approaches for increasing the dissolution of drugs include formation of salts, use of solubilizing agents, complexing agents, etc. Previously, there was considerable difficulty with formulating a large number of compounds using conventional approaches. Currently, the use of conventional approaches is increasingly limited due to side-effects associated with salt formation, addition of co-solvents and requirements of large quantities of excipients in the formulation (1, 6). Other methods to improve the bioavailability of drugs include microemulsions (7), inclusion of cyclodextrins (8), melt extrusion (9), emulsions (10), liposomes (11), and solid dispersions (2, 6). All these methods have been successfully utilized in developing formulations for poorly water soluble drugs, especially for compounds which are highly potent. In addition to the above mentioned approaches, methods such as particle size reduction to the sub-micron size range, e.g. nanosizing of drug particles or nanosuspensions, have gained much attention in recent years (2, 4, 12).

Nanosizing is defined as a pharmaceutical process that involves reducing the particle size of the active pharmaceutical ingredient to the nanometre size range. It means achieving the particle size below the sub-micron range i.e. particle size less than 1µm (13). Nanosizing techniques have become more popular as they can be applied to most compounds which have poor solubility issues. These techniques are referred to as ‘non-specific techniques’ to improve the bioavailability of poorly soluble drugs (13-15). According to the Noyes-Whitney

equation, a decrease in the particle size of a drug results in an increase in its surface area and thus the dissolution rate will increase proportionally which results in better absorption of poorly soluble drugs (3, 4, 13). Recent advancements in nanosizing techniques have enabled the pharmaceutical industry and researchers to produce particles in the 100-200 nm range in a reproducible manner (3).

There are various approaches for nanosizing drugs and these are classified as top-down, bottom-up and combination approaches. Top-down techniques involve particle size reduction using high energy approaches such as media milling and high pressure homogenization. Currently, there are six FDA approved nanocrystal drugs in the market which have been prepared by top-down techniques (16). All these processes are conducted in a liquid medium and thus they form nanosuspensions which are later processed into capsules or tablets or marketed as suspensions (3, 17).

Nanosuspensions refer to colloidal dispersions of sub-micron drug particles which are stabilized by addition of a suitable polymer or surfactant, and are of a particle size below 1000 nm (4, 13, 18). The dispersion medium can be aqueous e.g. water, or non-aqueous e.g. liquid polyethylene glycol and oils (13). Bottom-up techniques are essentially a precipitation technique as the nanosized drug particles are obtained after precipitation from a supersaturated drug solution (16). Bottom-up techniques offer many advantages such as being low energy processes and less expensive in comparison with other nanosizing methods, and produce particles with narrow size distribution. However, very few products prepared by bottom-up techniques have made it to market (19). Bottom-up techniques have recently been used in combination with top-down techniques to obtain even smaller particles.

Although these techniques have been in use for at least a decade, very few nanocrystals with a particle size of 100 nm have been obtained. Various attempts have been made to develop particles of less than 100 nm as it has been reported that drug nanocrystals of less than 100 nm have novel physical properties and show improvement in permeation through various biological barriers (16). Drug nanocrystals have improved the bioavailability of poorly-soluble drugs that are administered through a variety of routes including oral, dermal, ocular, buccal and pulmonary.

This review article will focus on various techniques for preparation of drug nanocrystals e.g. bottom-up techniques, top-down techniques and combination techniques. Case studies will

illustrate the ways in which the particle size of certain drugs has been reduced to the sub-micron range by utilising various nanosizing techniques.

2. Biopharmaceutical classification system (BCS)

The BCS is a system which classifies drugs according to their aqueous solubility and intestinal permeability. Aqueous solubility correlates with *in vitro* dissolution and the intestinal permeability correlates with *in vivo* bioavailability of the drug particles (6). According to the BCS, a drug is considered highly soluble if the highest strength of the drug is soluble in 250 ml of water and it is considered highly permeable if intestinal permeability of the drug is 90%. On the basis of BCS, all drugs have been classified into four categories i.e. BCS class I, class II, class III and class IV (6). The BCS System classification is shown in table 1 below:

Table 1. BCS Classification (6)

BCS Class I (high solubility and high permeability) e.g. Metoprolol, Diltiazem	**BCS Class II** (low solubility and high permeability) e.g. Phenytoin, Danazol
BCS Class III (high solubility and low permeability) e.g. Acyclovir, Cimetidine	**BCS Class IV** (low solubility and low permeability) e.g. Furosemide, Hydrochlorothiazide

The drugs in BCS class II and class IV have low solubility, therefore nanosizing of these drugs can increase their bioavailability. The BCS classification has been well accepted but a revised classification system, known as the Developability Classification System (DCS) has recently been introduced to classify drugs in a more relevant manner and it has been particularly useful in predicting critical factors related to *in vivo* performance as compared to BCS (20). In some cases it has been found that drugs have such a low aqueous solubility that even the nanocrystals of that drug showed very low bioavailability (21). For this reason, DCS classifies drugs according to whether they have dissolution rate-limited, solubility-limited or permeability-limited bioavailability. The dissolution rate-limited drugs are categorized under DCS class IIa and solubility-limited drugs are classified under DCS class IIb. Nanosizing of

drugs has been found to be a suitable approach for dissolution-rate limited compounds i.e. DCS class IIa (21).

3. Methods of nanosizing:

As previously described, there are different nanosizing methods and these are classified as bottom-up techniques, top-down techniques and combination techniques (4).

4. Bottom-Up Techniques

Bottom-up techniques are also referred to as precipitation techniques as nanosized drug particles are formed by precipitation which can be in crystalline or amorphous form (13). In this method, drug is precipitated from supersaturated drug solution, or by evaporation of solvent, or by mixing the drug with a non-solvent (4, 14). These techniques have not gained much traction in the pharmaceutical industry due to difficulties in controlling particle growth and the process (21). However, bottom-up techniques are used in combination with top-down techniques to improve the effectiveness of the method. Some bottom-up technologies used are sonocrystallization, confined impinging liquid jet precipitation, high gravity controlled precipitation technology, and multi-inlet vortex mixing (13).

4.1 Precipitation by addition of liquid antisolvent

Precipitation of a drug from its solution by addition of an antisolvent is an effective method of obtaining nanosized particles. Moreover, the size of the particles and morphology of the finished product can be controlled (24). In this method, a drug is dissolved in a solvent and then mixed with another solvent which is miscible with the first solvent but acts as an antisolvent (e.g. water) for the drug (16, 25). This results in increased super-saturation of the solution due to diffusion of solvent into the antisolvent and nucleation of the particles. During drug precipitation, particle growth and nucleation can compete by a process called "Ostwald ripening" but the process should be directed towards nucleation by the use of suitable excipients and stabilizers (24, 26).

This method of obtaining nanosized drug particles is easy and cost-effective as compared to other methods of nanosizing (27, 28) and it can be easily scaled up as it does not require any expensive equipment (29). There are, however, some critical process parameters that affect the particle size and the physicochemical properties of the nanosized drugs such as the nature

and selection of solvent and antisolvent, volume ratio of solvent and antisolvent, and their order of addition (16). An antisolvent precipitation technique has been used to prepare drugs such as atorvastatin (30), taxifolin (31), amorphous amphotericin B (32), and danazol (33). This method of nanosization can be divided into two types i.e. simple mixing and modified mixing methods (16).

4.1.1. Simple mixing method using static mixer

In this method, nanosized drug particles are produced by mixing the drug solution and antisolvent using mixing forces. The solvent plays an important role in production of submicron particles as it should solubilize the drug and have a fast diffusion rate towards the antisolvent. The solvents used for preparation of drug solution can be organic e.g. ethanol, methanol, IPA, NMP, acetone etc., or co-solvents e.g. polyethylene glycol or propylene glycol (16, 34). Solvents such as PEGs which are 'environmentally friendly' are preferred over other organic solvents which are toxic in nature (16). Selection of solvent is based primarily on the solubility of drug in the solvent but other factors such as the interaction between solvent and stabilizer are considered important (16). Static mixers have been described in the literature as 'simple mixing equipment' (35, 36). Static mixers offer several advantages over other mixing equipment including low cost, low energy consumption, less space required and homogenous mixing of miscible solvents (37).

Nanomorph® technology for the preparation of nanosupensions was developed by SOLIQS (Abbott GmbH & Co., KG, Ludwigshaven, Gemany) and Hydrosols prepared by Sucker (Novartis) are examples of antisolvent precipitation techniques (26, 38). These techniques can be employed for continuous operations in the pharmaceutical industry instead of batch production processing (38). The process of preparing drug nanoparticles by the Nanomorph® technique involves dissolving a drug in a suitable organic solvent then mixing the resultant solution with an antisolvent containing stabilizers; this means that precipitation of drug takes place in presence of stabilizers (16, 26). Stabilizers or polymers prevent the growth of coarse particles or aggregation of particles and help to keep the drug particles in their nanoparticle stage (39).

4.1.2. Modified Mixing Methods

These methods involve precipitation of drug by additional external features. It has been reported, for example, that addition of ultrasonic waves or alteration in the environment of

precipitation e.g. freeze drying, spray drying or high gravity precipitation, may produce smaller particles compared to conventional methods (16).

4.1.2.1. Sonoprecipitation

Ultrasonic sound has been used to provide homogenous conditions within the vessel during the antisolvent process (40). The principle behind sonoprecipitation is formation of bubbles, a process known as cavitation, and simultaneous collapse of these bubbles which releases shock waves. The waves result in faster and more uniform nucleation which further results in smaller particle size and reduction of agglomeration of particles by controlling the number of nuclei (40, 41). The process has been reported to increase micro-mixing and formation of amorphous particles with uniform particle size (16, 40). However, the final product particle size is dependent on the duration and intensity of sonication, the frequency of ultrasound, horn length (16).

The sonoprecipitation process is very fast and results in homogenous mixing of solvent and antisolvent within seconds after the application of ultrasonic sound (40). As the process is very fast, less organic solvent is required for antisolvent precipitation (41). The experimental set-up for sonoprecipitation is very simple and it consists of an ultrasonic bath containing antisolvent and solvent atomized into the sonicator using compact air pressure (16). Sonoprecipitation techniques have been used to prepare cefuroxime axetil nanoparticles with a particle size of 130 nm, and it was found that by doubling the amplitude of sonication, particle size was further reduced to 80 nm (41).

4.1.2.2. High gravity controlled precipitation technique (HGCP)

This technique has been used for the preparation of nanosized inorganic particles e.g. $CaCO_3$ (15-40 nm) and $Al(OH)_3$ (1-10 nm) (16, 42), and organic particles e.g. salbutamol sulphate, ephedrine and benzoic acid (43-45). The drug particles obtained by this method were found to have a narrower size distribution and specific morphology and crystal state as compared with particles produced by other methods (42). The HGCP technique utilizes a rotating packed bed containing packing material made of metal or plastic wire mesh (Figure 1). Two liquid streams (reactant A and B) pass via distributors and are mixed at the centre of the rotating packed bed where the mixture is subjected to high gravity due to centrifugal forces which force the mixture to pass from the packing before leaving the reactor (41).

The HGCP technique can be combined with the antisolvent precipitation process and is known as the high gravity antisolvent precipitation (HGAP) process (16). This process has been used in the preparation of cefuroxime axetil nanoparticles with a mean particle size of 300nm without using stabilizers. The particles were reported to have a high specific surface area which was around four times higher than that of commercial cefuroxime axetil (46). The HGAP process has also been utilised to prepare nanoparticles of other pharmaceutical ingredients such as danazol with a particle size of about 190 nm (33). Figure 1 shows a schematic diagram of high gravity controlled precipitation (HGCP).

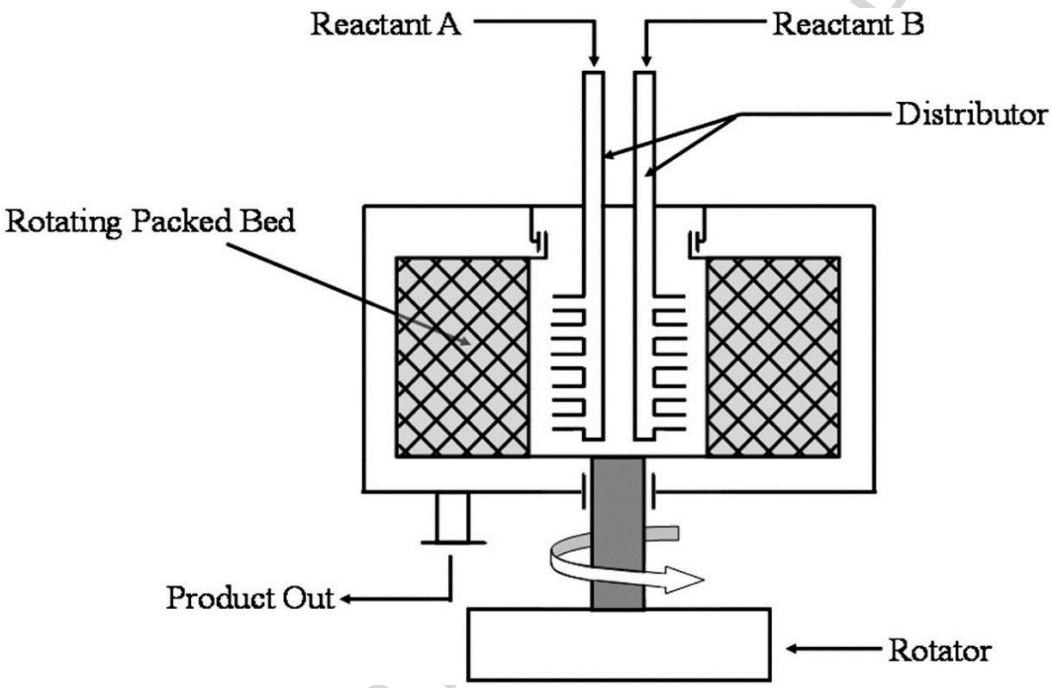


Fig. 1. Schematic diagram of HGCP (41).

4.2. Supercritical fluid (SCF) technique

Supercritical fluids have unique physical properties including low density and viscosity and have a high diffusion rate which helps to attain rapid mixing for precipitation (41). Carbon dioxide ($CO_{2)}$ is the most favourable SCF used for pharmaceutical processing due to its almost ambient critical temperature of 31.1°C and pressure of 72.9 atm which allows it to be easily converted to a supercritical state (16, 41). Moreover, it is inexpensive, readily available and non-toxic. Supercritical fluid techniques that have been used by pharmaceutical companies and researchers include rapid expansion of supercritical solution (RESS) and supercritical antisolvent (SAS) (16, 41).

4.2.1. Rapid expansion of supercritical solutions (RESS)

This technique has been employed for drugs which have good solubility in CO_2 (47). As CO_2 has a low polarity, it dissolves hydrophobic drugs to form a solution which rapidly expands into a low pressure area through an opening of a narrow nozzle (41). As the solution reaches a low pressure area, its density changes which results in supersaturation of the solution and thus precipitation of solute (16). One of the major drawbacks of this technique is that many drugs are not soluble in supercritical carbon dioxide and, to overcome this problem, a modified process known as rapid expansion of supercritical solution with solid (RESS-SC) has been introduced. In the RESS-SC method, a solid co-solvent is added to increase the solubility of the polar compounds (16). The other RESS modified processes are rapid expansion of supercritical fluid into a liquid solvent (RESOLV), and rapid expansion from supercritical to aqueous solution (RESAS) (16, 47). Table 2 shows examples of nanoparticles obtained by RESS and modified RESS techniques.

Table 2. Summary of nanoparticles obtained by RESS and modified RESS techniques.

API	**Method**	**Solvent**	**Operating conditions**	**Mean diameter (nm)**	**Reference**
Raloxifene	RESS	CO_2	100-180 bar 40-80°C	19nm	(48)
Clobetasol propionate	RESS-SC	CO_2 and menthol (co-solvent)	200-260 bar 70-110°C	95nm-318.8nm	(49)
Theophylline	RESS-SC	CO_2 and vanillin (co-solvent)	220 bar 40-70°C	85nm	(50)
Megestrol acetate	RESS-SC	CO_2 and menthol (co-solvent)	150-250 bar 40-60°C	102.8-516.3nm	(51)
Retinyl palmitate poly(L-lactide)	RESOLV	CO_2	330 bar 70-100°C	40-110nm	(52)
Naproxen	RESS	CO_2	Up to 400	560-820nm	(53)

			bar 26-327°C		
Phenytoin	RESS-SC	CO_2 and menthol (co-solvent	96-196 bar 45°C	75-120nm	(54)

4.2.2. Supercritical Antisolvent (SAS)

Supercritical fluids (SCFs) have gained significant attention in the last decade. SCFs have been used for processing of particles in the food, cosmetic and pharmaceutical industries (55).The technique uses the unique properties of supercritical fluids and is especially useful for those compounds which are insoluble in such fluids. In such cases, supercritical fluids act as an antisolvent and precipitate the drug from the drug solution (41). The method involves dissolving a drug in an organic solvent which is miscible with supercritical antisolvent to prepare a solution (16). The drug is then precipitated from solution either by adding supercritical antisolvent or drug solution into supercritical antisolvent (16, 41). In both cases, solvent diffuses into the antisolvent phase because of its miscibility with antisolvent and drug precipitation occurs due to low solubility into the SCF.

Different types of nozzles are available to introduce the drug solution into SCFs including single or co-axial nozzles (41). The particle size obtained after precipitation of the drug depends on factors such as the type of solvent/antisolvent, volume ratio, concentration of drug in the solution, the degree of mixing, and the rate of antisolvent addition (16). In addition, factors such as temperature, pressure and diameter of the expansion nozzle also affect the final particle size. It has been reported that a smaller particle size can be obtained by using a narrow nozzle and by increasing the pressure and decreasing the temperature of the supercritical antisolvent (16). A number of antisolvent processes have been used for the production of nanoparticles including gas antisolvent (GAS) (56), aerosol solvent extraction system (ASES) (57), solution enhanced dispersion by supercritical fluids (SEDS) (58) and SAS with enhanced mass transfer (SAS-EM) (59).

4.3. Solvent Removal Techniques

These techniques are based on removal of solvent. Conventional methods include spray-drying and freeze-drying, however neither of these techniques can produce nanoparticles as more effective liquid atomization is required. Both techniques are used with improved atomization to achieve nanoparticles (16).

4.3.1. Nano spray drying technique

The Nano spray dryer B-90 was developed by Buchi Labortechnik AG, Switzerland (60). Figure 2 shows the structure of the nano spray dryer. This dryer has been designed to provide particle size within the range of 300nm to 5µm. The principle of the technique is that drying gas enters the drying chamber from the top and gets heated to the set inlet temperature. The piezodriven spray nozzle forms very fine droplets which are gently dried in the drying chamber. The dried particles are then collected on the collecting electrode (61). The drying gas exits the spray dryer from the bottom outlet after being filtered before leaving the dryer. Both the inlet and outlet temperatures are measured after heating and filtration (60). The ultra-fine droplets are generated through vibration technology. The piezoelectric actuator which is driven at an ultrasonic frequency of 60 KHz results in vibration of a stainless steel membrane or mesh (4.0 to 7.0 µm) and thus ejects ultra-fine droplets every second (61). The particles obtained by this method have a narrow particle size distribution (60).

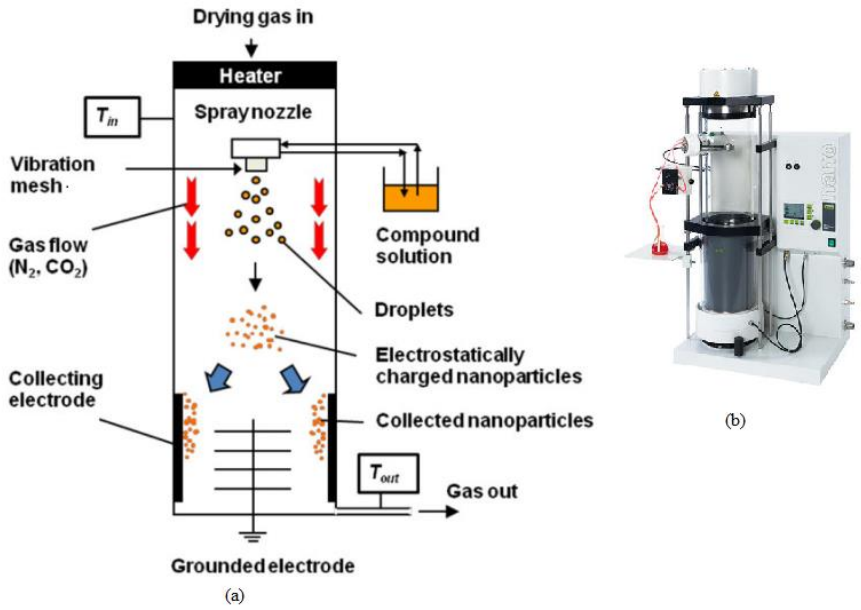


Figure 2. (a) Schematic diagram of Nano Spray Dryer (62), (b) Buchi Nano Spray Dryer B-90 (61)

Recently, Venugopala et al. prepared nanocrystals of vildagliptin using the Buchi Nano Spray Dryer B-90. The nanosized drug particles obtained had a narrow size distribution with a particle size of 445 nm (63). In another study, the nano spray dryer B-90 was used to obtain sub-micron size particles of calpain inhibitor. The particles obtained were in the range of 300 nm to submicron metre (62)

4.3.2. Spray Freezing into Liquid (SFL)

In this method, a solution containing drug and excipient is atomized into the cryogenic liquid which results in formation of frozen particles (64) that are then immediately collected and lyophilized. The process of atomization with rapid freezing results in the formation of amorphous nanoparticles with enhanced solubility and high surface area (64). Hu et al. prepared rapidly dissolving danazol powder with a diameter of 100 nm using this method. The particles were reported to have a very high surface area and solubility as 95% of danazol particles formed through the SFL process dissolved within 2 minutes (64). For large scale production by this method, the mixing step was modified using a 3-way nozzle i.e. different nozzles for solvent, antisolvent and atomizing air (65). The solvent and antisolvent are pumped into the nozzle through separate channels then mixed as soon as they leave the nozzle. The air, which is supplied by a third channel, facilitates the thorough mixing of solvent and antisolvent. The formed mixture enters into liquid nitrogen and is frozen at a faster rate than in the batch process (65). The particles obtained by this modified process have a smaller size and a higher dissolution rate (65).

5. Top-Down Techniques

Top-down techniques have become increasingly recognised by the pharmaceutical industry and there are currently a few marketed preparations which have been prepared by top-down techniques as shown in Table 3. Top-down techniques are high energy processes which are used to break down the particle size of drugs to the nanometre size range by application of friction. The two main top-down approaches are high pressure homogenization and media milling. These two techniques have been widely utilised by the pharmaceutical industry as they are easy to scale up to a commercial level and have also been accepted by regulatory authorities (5, 21, 66).

Table 3. Nanocrystals prepared by top-down techniques (19, 21, 23).

Brand Name	Active Ingredient	Company	Method	Technology	Administration route
Rapamune®	Sirolimus	Wyeth	Top-down/WBM	Nanocrystal®	Oral
Emend®	Aprepitant	Merck	Top-down/WBM	Nanocrystal®	Oral
Tricor®	Fenofibrate	Abbott	Top-down/WBM	Nanocrystal®	Oral
Triglide®	Fenofibrate	Skyepharma	Top-down/HPH	IDD-P®	Oral
Megace®ES	Megestrol acetate	Par Pharmaceuticals	Top-down/WBM	Nanocrystal®	Oral
Invega® Sustenna ® Xeplion®	Paliperidone palmitate	Janssen	Top-down/WBM	Nanocrystal®	Parenteral

5.1. Media Milling

This technique was developed by Liversidge et al. in 1992 and is known as Nanocrystal® technology; it is regarded as one of the most successful nanosizing techniques (21, 67). Media milling techniques are the current versions of wet ball milling techniques. Wet ball milling techniques involve low energy ball milling in which a jar is filled with a milling medium and the drug dispersion in either aqueous or non-aqueous media is subjected to a milling operation (5, 21).

The milling media used for wet ball milling is usually glass beads and these are moved by either electric stirrer or by movement of the whole jar. The liquid dispersion medium prevents adhesion and compaction of drug particles on the walls of the jar and also provides lubrication for the newly formed particles. Low energy ball milling usually takes a long time and cleavage of particles occurs through fracturing, cleavage and abrasion (21). The current Nanocrystal™ technology media milling technique involves high energy wet ball milling.

High energy media milling requires suitable equipment and new media mills have been designed to meet the requirements of the pharmaceutical industry. These mills have high power densities so that production times can be reduced significantly and have the capacity to process up to 1000 kg of active ingredients per batch. In this process, the drug needs to be

exposed to high energy for 30 to 120 minutes which results in a high quality nanosuspension (2, 5, 12, 21). The media milling process is similar for small scale to commercial scale operations and can be operated in batch mode (discontinuous mode) or re-circulation mode (continuous mode).

The commercial media milling process takes place in continuous mode and the media mills are equipped with media separators, which hold the milling medium within the milling chamber while the nanosuspension is in the circulation mode. The suspension of poorly water soluble drug is added to the tank of the media milling chamber and, from this chamber, the suspension is pumped to the milling chamber where the particle size is reduced to nanometre size range by the fracturing, abrasion and cleavage mechanisms (2, 12, 21).

The movement of balls in the milling chambers is initiated by a stirring device i.e. a central shaft with several discs, and rotates at a speed of 20,000 rpm or above (5). The residence time for achieving a particle size <200 nm varies from 30 to 120 minutes, depending on the properties of the compound and the extent of particle size reduction. As media milling is a continuous process, there is a screen at the exit of the milling chamber which separates the suspended, milled particles from the milling media. It has been reported that there is a grinding limit for drugs under a set of conditions such as milling speed, temperature and time, whereas the input of energy does not affect the particle size of drug (2).

The media milling process requires special media and is selected on the basis of the inner surface of the milling chamber and the type of agitator used. Previously, glass beads or zirconium oxide beads were used but these resulted in contamination of the nanosuspension by abrasion with the milling chamber. Later, highly cross-linked polystyrene beads e.g. Pollymill® were introduced to minimise the contamination in the final nanosuspension. Pollymill® media of highly cross-linked polystyrene 0.5mm diameter beads (2, 5, 21) produce high quality nanosuspensions which are suitable for parenteral administration.

Recently, the milling media used for the nanosization of drugs have been of much smaller dimensions i.e. <100 µm and centrifugal techniques have been used instead of conventional screens to separate the milling media from the milled product. The Ultra Apex Mill is an example of this type of machine where the centrifugal technique is used with media milling to separate the milling media and the milled product (5). A diagram of a media mill is shown in figure 3 below:

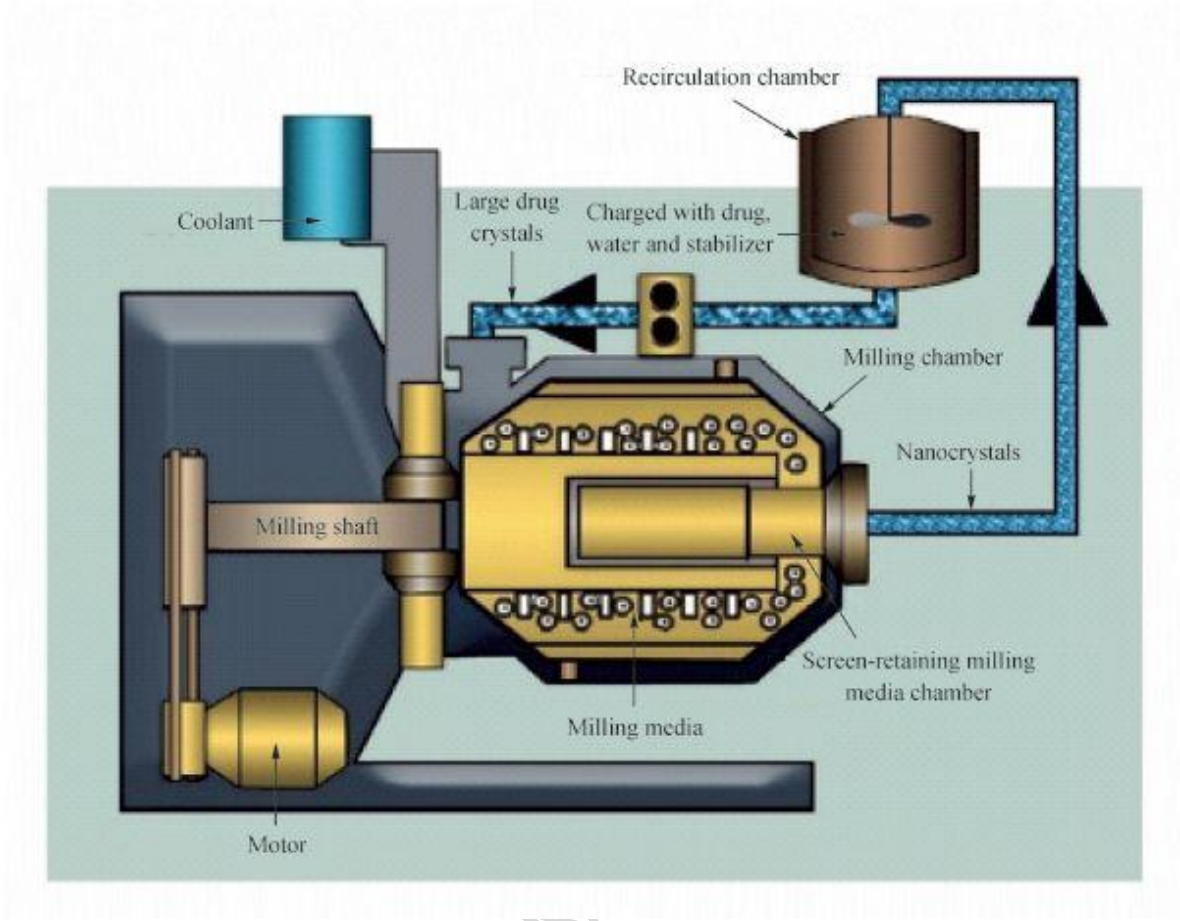


Figure 3. Schematic diagram of Wet Media Milling Process (68)

Commercial equipment is available for both small scale and large scale production of nanocrystals. For example, agitated ball mills can be used for production of nanosuspensions of drug quantities as low as 10 mg. Commercial media milling equipment for large scale production is also available. The media milling process has produced smaller particles than other top-down techniques (21) It is, however, important to analyse the final nanosuspension for any impurities which may occur due to the milling chamber or milling media.

The media milling process can be optimised by selecting the mediium so that it can withstand the process and by controlling process parameters such as milling time and shaft speed. The resultant nanosuspensions can be formulated into other dosage forms including tablets or powders. Such nanocrystal techniques have been accepted by regulatory agencies worldwide (2, 5). Table 4 shows examples of nanocrystals obtained by wet ball milling techniques.

Table 4. Nanocrystals prepared by wet media milling technique

No.	Drug	Particle size obtained	Milling Time	Equipment used	Reference
1.	Fenofibrate	150 nm	120 min	Wet-stirred media mill (Netzsch Fine particle size technology, PA, USA)	(69)
2.	Griseofulvin	<100nm	64 minutes	Microcer stirred media mill (Netzsch Fine particle size technology, PA, USA)	(70)
3.	Bifendate	120 nm	45 minutes	Mini-easy nano fine mil (Retch Topway Technology Co., Beijing, China)	(71)
4.	Phenytoin	292 nm	90 minutes	Oscillating beads-milling apparatus (Multi-beads Shocker, Osaka, Japan)	(72)
5.	Miconazole	140 nm	60 minutes	High energy mill (Labstar LS1 Minicer, Netzsch, Germany)	(73)
6.	Itraconazole	136 nm	60 minutes	High energy mill (Labstar LS1 Minicer, Netzsch, Germany)	(73)
7.	Naproxen	200 nm	240 minutes	Netzsch media mill (NETZSCH, Blandon, PA)	(74)

5.2. High Pressure Homogenization (HPH)

This method for the production of nanosuspensions was patented by Muller et al. in 1994 and is considered the second most important technique for the production of drug nanocrystals (75). The HPH method employs two types of technique i.e. the microfluidization technique and Piston-gap homogenizers. The microfluidizer technique was developed by Skyepharma and it is known as the IDD-P™ technique i.e. insoluble drug delivery micro particle technology. This technique is based on the principle of jet-stream homogenization where the drug suspension is homogenized at high velocity and pressure by a homogenizing chamber.

In this technique, two types of chambers are used i.e. the 'Y' type chamber and 'Z' type chamber. In the 'Z' chamber, the direction of flow of suspension changes which results in particle collision and shear forces and finally reduction in particle size. In the 'Y' type chamber, the suspension flows in two streams which collide with each other resulting in particle collision and the particles break because of shear and cavitation forces. It has been reported that due to the low power density of this system, 50 or more passes are required through the microfluidizer to obtain the required particle size. Thus, it is not regarded as a 'production friendly' method (5, 21, 66).

The piston-gap HPH technique developed by Muller et al. was termed Dissocubes™ and currently this trademark is owned by Sky Pharma. A second variant of piston-gap HPH was developed in 1999 and is known as Nanopure® technique and it is owned by Pharmasol GmbH/Berlin. The technique involves homogenization of drugs in either a medium without water or with a reduced water content e.g. a mixture of water with PEG.

5.2.1. Dissocubes Homogenization Technique

In this technique, the drug is mixed in an aqueous solution of surfactant and stabilizers and the resulting dispersion is passed through a narrow gap (diameter 25 um) in the homogenizer with a very high velocity and a very high pressure of 500 to 1500 bars. Before entering the narrow gap, the dispersion is present in a cylinder which has a diameter of about 3 cm (5, 21, 66, 76). The suspension of drug is passed through the homogenizer repeatedly to achieve the desired particle size and pressure is increased from 500 bar to 1500 bar in a stepwise manner. As the width of the gap is very narrow i.e. a few micrometres, it is recommended that the starting material is pre-micronized using fluid energy milling to prevent clogging in the gap and to reduce milling time (21).

When the drug mixture is contained in a closed system, the pressure is constant but as soon as the mixture passes through a narrow gap, an increase in the dynamic pressure of the liquid develops and the static pressure of the liquid in the gap falls below the vapour pressure which results in boiling of the liquid and formation of bubbles. As soon as the mixture leaves the homogenization gap, the gas bubbles collapse due to the normal pressure conditions and drug particles of nanometre size range are achieved by cavitation forces (which are generated because of formation and collapse of gas bubbles), shear forces and collision.

The quality of the resultant drug particles depends on various parameters including the power density of the system, the number of homogenization cycles, the hardness of the drug, and temperature (5, 76). HPH can be used for both laboratory and commercial applications as it is a scalable process. HPH does not generate impurities due to abrasion or cleavage thus, it can be used to produce parenteral nanosuspensions. In addition, the latest homogenizers have ceramic valves which can produce nanosuspensions without impurities even if there are harsh processing conditions (21). Figure 4 shows a schematic diagram of a high pressure homogenizer.

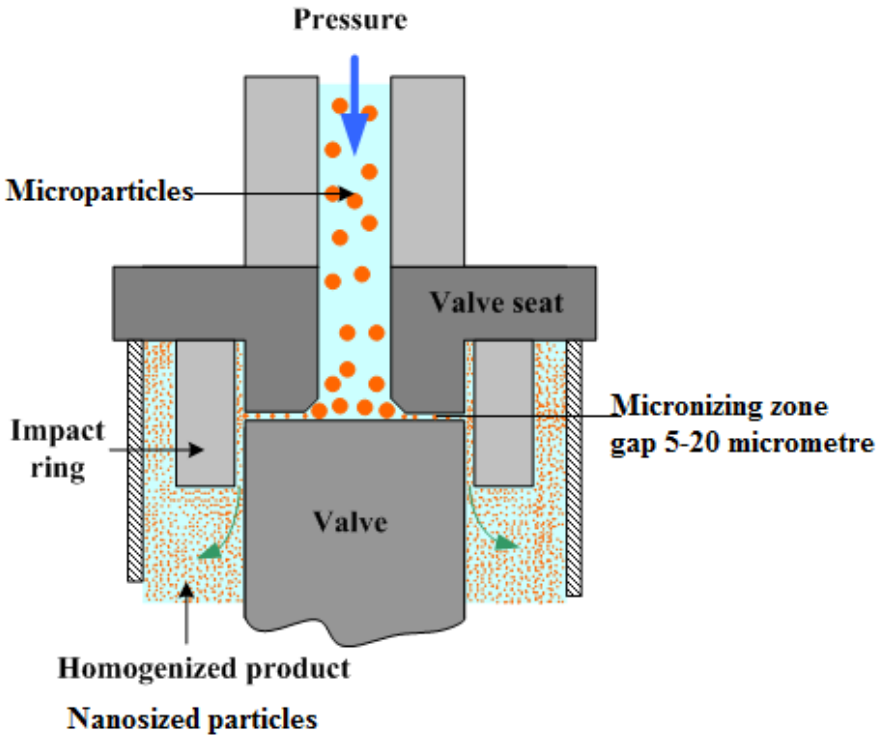


Figure 4. Schematic diagram of high pressure homogenizer (77)

5.2.2. Nanopure® Technology

The most important feature of this technique is that the process can take place a in non-aqueous phase (e.g. polyethylene glycol) or a reduced water phase (water-alcohol mixtures), thus it is suitable for reducing the particle size of thermolabile drugs and drugs which undergo hydrolysis. It is also used when nanosuspensions are converted into solid dosage forms such as tablets since less heat will be required for removing solvent from the system as compared to homogenization with water. In this method, drug powder is mixed in a non-aqueous medium or a reduced-water mixture as described above and the resulting suspension is homogenized in a piston-gap homogenizer. This type of homogenization is performed at low temperatures e.g. at 0°C and below freezing point e.g. -20°C. It was found that homogenization was more effective at low temperatures. The shear forces of the turbulent flow help to break down the drug particles to the nanometre size range (5, 76, 78).

Recently, supercritical CO_2 has been utilized as a dispersion medium in the homogenization of ketoprofen and phenytoin (5). Nanopure technology is available commercially for lab scale as well as commercial scale production e.g. Micron Lab 40 is a machine used by PharmaSol, GmbH, Germany. The homogenizer allows pressure to vary from 100 bars to 1500 bars and it also contains a temperature-controlled jacket which maintains the temperature at 0°C or below and can be used for 40 ml samples. A homogenizer used for a large scale production by the pharmaceutical industry is the Rannie 118; it can process materials up to 1200 L per hour at a pressure of 1500 bar (78). Table 5 shows examples of drug nanocrystals which have been produced by high pressure homogenization.

Table 5. Examples of drug nanocrystals obtained by high pressure homogenization

No.	API	Particle size	Operating Conditions	Equipment used	Reference
1.	Quercetin	338.3 nm	2 cycles at 300 bar, 2 cycles at 500 bar, 1 cycle at 1000 bar and 20 cycles at 1500 bar	LAB 40 high pressure homogenizer (APV Deutschland GmbH, Unna, Germany)	(79)
2.	Atorvastatin	215.3± 14.2 nm	1-3 cycles at 1378 bar to 2757.9 bars	High-pressure homogenizer (Nano DeBEE, BEE International Inc., MA, USA)	(80)
3.	Budesonide	640 nm	60 cycles at 1000 bar	High pressure homogenizer (AH110D, ATS Engineering, Italy)	(81)
4.	Itraconazole	267.6±15.8 nm	2 cycles at 150 bar, 2 cycles at 500 bar, 2 cycles at 1000 bar and 15 cycles at 1350 bar	AH100D high pressure homogenizer (ATS Engineering Inc., China)	(82)
5.	Herpetrione	286±1.3 nm	5 cycles at 500 bar and 20 cycles at 1000 bar	High shear homogenizer (Ultra-Turrax T25, IKA, Germany)	(83)
6.	Nimodipine	450nm	2 cycles at 200 bar, 5 cycles at 500 bar then 15-20 cycles at 1500 bar	Niro-soavi NS1001L (ATS Co. Ltd., Italy)	(84)

6. Combination Techniques

Various combinations of precipitation techniques and high energy processes have been used to prepare drug nanoparticles. Using these combination approaches, significant reductions in processing times can be achieved by subjecting the drug to precipitation before processing through a top-down process (21). These techniques also help to achieve a better size reduction as compared to conventional techniques. The first combination approach was developed by Baxter and named Nanoedge™ technology (21, 67) The technique involved precipitation of a drug and then an annealing step is performed by application of high energy e.g. HPH. Annealing is a process of converting thermodynamically unstable matter into a stable form by applying stress followed by thermal relaxation (67). Combination techniques currently used include Nanoedge™, H69, H42, H96 and the CT combination technique (85)

6.1. Nanoedge™ Technology

Nanoedge technology is a combination of a micro-precipitation technique followed by a top-down technique such as high pressure homogenization (HPH). The drug is dissolved into an organic solvent (miscible with antisolvent) to form a solution which is then mixed with an antisolvent such as water. As the drug has the least solubility in water, the solvent quickly diffuses into the antisolvent causing the drug to precipitate. This step is a pre-treatment step and the amorphous or crystalline drug particles obtained by this method are subjected to HPH to make them thermodynamically stable by annealing (85). This technique has been used for production of the anti-cancer drug paclitaxel with improved solubility and small particle size (1000 nm) (85), and for formulation of itraconazole and isradipine nanosuspensions (16). The technique has also been used for preparation of ultrafine particles of beclomethasone dipropionate with a size range of 200 to 300 nm. It was found that aerosol performance was improved with the modified nanosized formulation as compared with original beclomethasone dipropionate (86).

6.2. H69 technology

This technology was developed by Muller and Moschwitzer and is a combination of the microprecipitation technique with HPH. It differs from the Nanoedge technique as cavitation occurs at the same time as particle formation (85). In this method, addition of antisolvent to drug solution is carried out in a controlled manner by using an infuser device. The

disadvantages of the H69 and Nanoedge techniques are similar as nanosuspensions prepared by both methods produce organic solvent residues which need to be further removed (85).

6.3. H42 technology

This technique was also developed by Moscwitzer (16) and involves a combination of spray-drying precipitation followed by HPH technique (87). The spray-drying process is a pre-treatment step which provides solvent free and fine drug particles which are further treated by the HPH process. Due to the nature of fine particles produced by spray-drying, the risk of clogging of the homogenizer is reduced (87). The H42 process has been used to obtain glibenclamide nanoparticles of particle size of about 236 nm (87). It has also been used for the production of nanoparticles of reseveratrol of mean particle size of 200 nm (85).

6.4. H96 technique

This techniques is used for production of nanosuspensions and involves freeze-drying as a pre-treatment step followed by high pressure homogenization. The precipitated drug particles are frozen by liquid nitrogen then freeze dried (85). The freeze-drying process eliminates the use of organic solvent and is optimised to produce brittle particles so that further processing with HPH yields a very small particle size. This technique has been used for the preparation of nanosuspensions of amphotericin B with a mean particle size of 100 nm (85).

6.5. Combination Technology (CT)

This is a combination of top-down techniques i.e. pearl milling and the high pressure homogenization process (88). The pre-treatment step is pearl milling of a macrosuspension followed by homogenization through HPH. The treatment with HPH provides physical stability to nanosuspensions by avoiding the Ostwald ripening phenomenon (85). The CT technique has been used for the processing of hesperidine nanosuspensions and a particle size of 599 nm was reported along with long term stability (85).

7. Stabilization of nanosuspensions

The nanosuspensions prepared by the methods described above offer many advantages such as improved drug bioavailability, high drug loading, reduced side effects , reduced dose and increase in patient compliance (18). Nanosuspensions reduce the risk of side effects as a lesser number of excipients are required for the formulation. Unfortunately, nanosuspensions can be unstable due to particle growth and nucleation. This is because nanosuspensions are

thermodynamically unstable due to their high surface area and the positive Gibbs free energy associated with a high surface area (18, 22).

7.1. Thermodynamically unstable nanosuspensions try to minimize the energy associated with the system by an agglomeration process (22). It has been found that the activation energy of the system plays an important part in the agglomeration process and stabilizing a nanosuspension can prevent the process by increasing the activation energy of the system (22). Therefore, addition of a suitable stabilizer to a nanosuspension is important to prevent aggregation of particles. Stabilizers induce ionic or steric stabilization by covering the surface of particles (89). Steric stabilization can be achieved by adsorbing the stabilizing polymer on the surface of drug particles and electrostatic stabilization can take place by adsorption of charged molecules or ions of stabilizing polymer or surfactant onto the surface of drug particles (90). . **Examples of stabilizers**

The stabilizers commonly used in the formation of nanosuspensions include polymers such as hydroxypropyl methyl cellulose (HPMC) (22), hydroxypropyl cellulose (91), polyethylene glycols (PEGs) (18), D-α-tocopherol polyethylene glycol succinate (92), polyvinyl alcohols (18), ionic surfactants like sodium lauryl sulfate (18), chitosan polymer (93) and non-ionic surfactants e.g. tweens (18). Ionic surfactants provide stabilization to nanosuspensions by electrostatic repulsion and polymers and non-ionic surfactants provide stabilization through steric repulsion. Some food proteins including soybean protein isolate and whey protein isolate have also been used as stabilizers. For example, He et al. prepared indomethacin nanosuspension using food proteins as stabilizers (18).

8. Conversion of a nanosuspension into solid products

Nanosuspensions can be stabilized using suitable stabilizers but their prolonged storage is associated with chemical instability resulting in their hydrolysis, chemical reactivity of the drug, or leakage of the drug. Conversion of a nanosuspension into a solid product can stabilize the product both physically and chemically. (22, 90). Freeze-drying and spray-drying processes have been used to transform nanosuspensions into dry powders. The dry powder can be further filled in capsules or compressed into tablets. However, the flow properties of the powder obtained by drying processes may change, resulting in poor flow, high bulk density and hygroscopicity. Suitable excipients are added to such powders to improve their flow properties.

In addition to spray-drying and freeze-drying, spray granulation and palletisation processes have also been used for obtaining dry powders (22). Rapid dissolution and disintegration are considered important parameters of solid dosage forms and both should be monitored while converting a nanosuspension into a solid dosage form. The dissolution potential of nanocrystals can be preserved by addition of matrix formers such as sucrose, lactose, mannitol and sorbitol before the drying step (94). Sucrose has been found to be a suitable matrix former and was found to enhance the dissolution rate of loviride nanocrystals when converted into a solid dosage form. Some examples of nanosuspensions dried by the above mentioned processes are shown in table 6 below:

Table 6. Examples of nanosuspensions dried by freeze-drying and spray-drying techniques.

No	**API**	**Drying Technique**	**Matrix used**	**reference**
1.	Itraconazole	Spray-drying	Mannitol	(73)
2.	Piroxicam	Freeze-drying	Mannitol	(95)
3.	Nifedipine	Freeze-drying	Sucrose, maltose, glucose	(96)
4.	Naproxen	Freeze-drying	None	(22)
5.	Budesonide	Spray-, freeze-drying	Mannitol	(97)

9. Case Studies

9.1. **Nanosizing of sodium ibuprofen by the Bottom-down technique (SAS method)** (55)

Mezzomo et al. 2015 produced nanosized ibuprofen sodium by the solvent-antisolvent method (SAS). Ibuprofen sodium was dissolved in acetone (solvent) by application of heat (40°C, 10 minutes) and agitation until ibuprofen was completely solubilized in acetone. The supercritical antisolvent used in the process was 99.9% pure CO_2 which was delivered as a liquid using a heat exchanger and at a pressure of 60 bar. A supercritical fluid extraction unit was used for the extraction of sodium ibuprofen and a pump was used to provide the desired pressure to the solvent and antisolvent. To start the experiment, pure carbon dioxide was pumped into the precipitator vessel and once the desired experimental conditions such as

temperature, pressure and flow rate of supercritical solvent were achieved, the drug solution was fed into the precipitator unit. Precipitated particles were stored at -10°C and were also protected from light.

The effect of temperature, pressure, solution flow rate and concentration of feed solution on precipitation of ibuprofen was evaluated at a constant flow rate of CO_2 i.e. 1 kg carbon dioxide per hour. The pressure ranged from 80 to 140 bar, temperature from 35 to 55°C, the solution flow rate was 1 to 3ml/min, and the concentration of feed solution varied from 0.5 to 1.5mg/ml. The resulting particles were evaluated in terms of morphology, size and degree of crystallinity using scanning electron microscopy (SEM), differential scanning calorimetry (DSC), Powder X-ray diffraction, and RAMAN spectroscopy. The results obtained by the SAS method were also compared with the results obtained by a conventional lyophilisation technique.

Phase equilibrium data showed that at 40°C ibuprofen was completely dissolved in acetone and addition of supercritical solvent to the drug solution resulted in the precipitation of ibuprofen at 35 to 45°C. From this experiment, it was concluded that CO_2 acts as an antisolvent and solubility of ibuprofen increases with an increase in temperature. Moreover, the morphology of precipitated ibuprofen was studied and compared with ibuprofen obtained by different operational conditions as well as the lyophilized samples and un-processed samples of sodium ibuprofen. It was observed that particle size of sodium ibuprofen was reduced to the nanometre size range (from 144±87µm to380±84 nm) by the SAS method. Moreover, the results obtained for lyophilized samples were smaller in size (126±25 µm) than unprocessed ibuprofen.

Overall, it was concluded from this experiment that nanoparticles of ibuprofen can be obtained by the SAS method using CO_2 as antisolvent. The particle size of ibuprofen was in the range of 380±84nm at all conditions of the experiment. However, the best operating conditions were found to be: Pressure 110 bar, temperature 35°C, concentration of ibuprofen 0.5mg/ml, flow rate 1ml solution/minute, and 1kg CO_2/hour. By reducing the ibuprofen particle size to the nanometre size range, the surface area can be increased and bioavailability can be improved significantly. Thus, the SAS method for the production of nanoparticles of ibuprofen was considered to be successful.

9.2. **Fabrication of quercetin nanocrystals: Comparison of different methods** (79)

Kakran et al (2012) studied different methods for fabrication of quercetin nanocrystals using HPH, bead milling and cavi-precipitation techniques (H69 techniques). The aim of the experiment was to compare the production efficiency, particle size and quality of products obtained. Quercetin is an antioxidant and several benefits of this compound have been reported such as protection against osteoporosis, some cancers, cardiovascular diseases, pulmonary diseases and ageing. This compound has been reported to have extremely low bioavailability (around 1%) in humans due to its poor solubility and high protein binding. Therefore, nanocrystals of the drug were prepared to improve its solubility and dissolution.

High Pressure Homogenization of 5% and 10% w/w quercetin suspension was performed using a LAB 40 homogenizer to produce a nanosuspension with a batch size of 40 ml. Nanosuspensions were made in ultra-purified water using tween 80 as a stabilizer (1% w/w and 2% w/w). Initially, a premilling step was conducted by increasing pressure (2 cycles at 300 bar and 500 bar each and then 1 cycle at 1000 bar). This was followed by HPH at 1500 bars for 20 cycles. In addition, another batch of samples was prepared at low pressure of 500 bar with 20 cycles. Samples were also collected after pre-milling and after certain homogenization cycles i.e. at 1, 5, 10, 15 and 20 cycles.

Another aqueous nanosuspension of quercetin was prepared by using a bead mill in a continuous mode. The milling medium used for the process was zirconia beads of size 0.4-0.6mm and 0.2mm. The milling chamber had a capacity of 200 ml and was filled with 150 ml of milling media and then 50 ml of quercetin suspension with 5 % and 10% quercetin. The nanosuspension was stabilized using tween 80 (1% and 2% w/w). The suspension was milled in the milling chamber for 90 minutes at a speed of 2000 rpm. Samples were collected at regular intervals during the milling process i.e. after 5, 30, 60 and 90 minutes.

In another experiment, a suspension of quercetin was prepared using the cavi-precipitation technique. In this method, DMSO and ethanol were used as solvents and 100 ml aqueous solution of tween 80 was used as the antisolvent. Quercetin solutions were prepared by dissolving in 20 ml DMSO and 50 ml ethanol. The solution was pumped at a rate of 1ml/min via an injection pump into the micro-fluidizer which contains antisolvent. The resulting nanosuspension was homogenized at 500 bar.

The nanosuspensions obtained by the above experiments were subjected to particle size analysis using photon correlation spectroscopy (PCS) and laser diffractometry. PCS was used to determine the mean particle size and polydispersity index (PDI). PDI gives an indication of

the size distribution of the samples. PDI values from 0.1 to 0.2 indicate a narrow particle size distribution, but PDI value of 0.5 indicates a broad distribution of particles. Laser diffraction was used for measurement of larger particles i.e. up to 2000µm. The morphology of particles was studied by field emission scanning electron microscopy. A solubility test of quercetin was undertaken in ultra-purified water (milli-Q water) in a water bath at a temperature of 25°C. Dissolution tests for quercetin were performed by using a USP II rotating paddle apparatus at a temperature of 37°C.

It was observed that as the number of homogenization cycles increased the particle size of of quercetin decreased with 20 cycles found to be optimal. Moreover, the particle size reduced with increased pressure, e.g. the particle size almost halved when pressure was increased from 500 bar to 1500 bar. The particle size of 5% and 10% w/w quercetin suspensions were 338.3 nm and 442 nm respectively.

There was no significant difference in the particle size of quercetin nanosuspensions obtained by using 0.2 mm and 0.4-0.6 mm beads for milling. The particle size obtained by using the smaller beads was slightly higher which suggests that decreasing the size of milling beads does not always result in greater particle size reduction. After 60 minutes of milling time using 0.4-0.6 mm milling beads, the average particle size obtained for 5% and 10% formulations were 253.2 nm and 270.7 nm respectively. Increasing milling time by an additional 30 minutes produced an increase in particle size due to an increase in kinetic energy of the particles which then resulted in particle aggregation.

The results obtained using the Cavi-precipitation process revealed that the particle size obtained with DMSO was 469.7 nm and the PDI was 0.111. The smallest particle size obtained using ethanol as solvent was 559.4 nm with a PDI of 0.180. The solubility study results showed a significant increase in the saturation solubility of quercetin nanocrystals as compared to the coarse quercetin powder. The saturation solubility of quercetin increased by 9 times, to 25.59±1.11 µg/ml for nanosuspensions as compared to coarse nanosuspensions which had a saturation solubility of 2.84 ±0.03 µg/ml. It was also found that samples prepared using ethanol showed very high solubility during the cavi-precipitation process and could be used in the final formulations.

The dissolution rate of quercetin nanocrystals was higher than for coarse quercetin. Complete dissolution for nanosuspensions obtained by the milling method occurred in 120 minutes. However, 70% dissolution was observed for nanosuspensions prepared by the

homogenization process at 500 bar, and only 10% dissolution for coarse quercetin microsuspension.

Comparing the samples obtained by the three different size reduction methods used in the study revealed that the maximum particle size reduction was obtained by the bead milling process, followed by HPH and then cavi-precipitation. The smallest size of quercetin obtained by the bead milling process was around 276.7 nm with a saturation solubility of 25.59± 1.11 µg/ml. The particle size of quercetin obtained by cavi-precipitation process was higher compared to other two processes.

10. Conclusion

The concept of nanosizing of poorly-soluble drugs to improve bioavailability has been widely accepted by the pharmaceutical industry and regulatory authorities. However, to date, relatively few preparations of drug nanocrystals have been marketed. This may be set to increase as there have been many advances in nanosizing technologies in recent years. Challenges with top-down techniques such as long milling times and abrasion issues have been resolved. Issues with bottom-up techniques including agglomeration, stabilization, residual solvent content and other process parameters to obtain nanosized particles are currently being studied and resolved. Recently, combination techniques have gained lot of attention and show promise in overcoming problems associated with individual drugs and single techniques. The literature indicates that there is continuous improvement in all of these techniques and more drugs with solubility issues are being considered for nanosizing. Moreover, with a better understanding of the biopharmaceutical characteristics of drugs, it is easier to select the best candidates for nanosizing. It is now evident from the DCS classification that compounds which show dissolution rate-limited bioavailability are potentially the best candidates for nanosizing.

References:

1. Kumar S, Shen J, Burgess DJ. Nano-amorphous spray dried powder to improve oral bioavailability of itraconazole. J Control Release. 2014;192:95-102.
2. Merisko-Liversidge E, Liversidge GG. Nanosizing for oral and parenteral drug delivery: A perspective on formulating poorly-water soluble compounds using wet media milling technology. Advanced Drug Delivery Reviews. 2011;63(6):427-40.
3. Kesisoglou F, Panmai S, Wu Y. Nanosizing — Oral formulation development and biopharmaceutical evaluation. Advanced Drug Delivery Reviews. 2007;59(7):631-44.
4. Sinha B, Muller RH, Moschwitzer JP. Bottom-up approaches for preparing drug nanocrystals: formulations and factors affecting particle size. Int J Pharm. 2013;453(1):126-41.
5. Loh ZH, Samanta AK, Sia Heng PW. Overview of milling techniques for improving the solubility of poorly water-soluble drugs. Asian Journal of Pharmaceutical Sciences. (0).
6. Khadka P, Ro J, Kim H, Kim I, Kim JT, Kim H, Prakash Khadka a,1, Jieun Ro a,1, a, Kim I Kim J, Kim H, Min Cho J, Yun G, Lee J. Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. Asian Journal of Pharmaceutical Sciences. 2014;9(6):304-16.
7. Chen Y, Tuo J, Huang H, Liu D, You X, Mai J, Song J, Xie Y, Wu C, Hu H. Optimized mixed oils remarkably reduce the amount of surfactants in microemulsions without affecting oral bioavailability of ibuprofen by simultaneously enlarging microemulsion areas and enhancing drug solubility. International Journal of Pharmaceutics. 2015;487(1–2):17-24.
8. Tønnesen HH, Másson M, Loftsson T. Studies of curcumin and curcuminoids. XXVII. Cyclodextrin complexation: solubility, chemical and photochemical stability. International Journal of Pharmaceutics. 2002;244(1–2):127-35.
9. Shah S, Maddineni S, Lu J, Repka MA. Melt extrusion with poorly soluble drugs. International Journal of Pharmaceutics. 2013;453(1):233-52.
10. Choi YK, Poudel BK, Marasini N, Yang KY, Kim JW, Kim JO, Choi HG, Yong CS. Enhanced solubility and oral bioavailability of itraconazole by combining membrane emulsification and spray drying technique. International Journal of Pharmaceutics. 2012;434(1–2):264-71.
11. Matloob AH, Mourtas S, Klepetsanis P, Antimisiaris SG. Increasing the stability of curcumin in serum with liposomes or hybrid drug-in-cyclodextrin-in-liposome systems: A comparative study. International Journal of Pharmaceutics. 2014;476(1–2):108-15.
12. Merisko-Liversidge E, Liversidge GG, Cooper ER. Nanosizing: a formulation approach for poorly-water-soluble compounds. European Journal of Pharmaceutical Sciences. 2003;18(2):113-20.
13. Junyaprasert VB, Morakul B. Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs. Asian Journal of Pharmaceutical Sciences. 2015;10(1):13-23.
14. Salazar J, Ghanem A, Müller RH, Möschwitzer JP. Nanocrystals: Comparison of the size reduction effectiveness of a novel combinative method with conventional top-down approaches. European Journal of Pharmaceutics and Biopharmaceutics. 2012;81(1):82-90.
15. Möschwitzer J, Müller RH. New method for the effective production of ultrafine drug nanocrystals. Journal of nanoscience and nanotechnology. 2006;6(9-10):3145-53.
16. Sinha B, Müller RH, Möschwitzer JP. Bottom-up approaches for preparing drug nanocrystals: Formulations and factors affecting particle size. International Journal of Pharmaceutics. 2013;453(1):126-41.
17. Moschwitzer J, Muller R, Thassu D, Deleers M, Pathak Y. Drug Nanocrystals-The Universal Formulation Approach for Poorly Soluble Drugs. DRUGS AND THE PHARMACEUTICAL SCIENCES. 2007;166:71.
18. Wang Y, Zheng Y, Zhang L, Wang Q, Zhang D. Stability of nanosuspensions in drug delivery. Journal of Controlled Release. 2013;172(3):1126-41.
19. De Waard H, Frijlink HW, Hinrichs WLJ. Bottom-up preparation techniques for nanocrystals of lipophilic drugs. Pharmaceutical Research. 2011;28(5):1220-3.

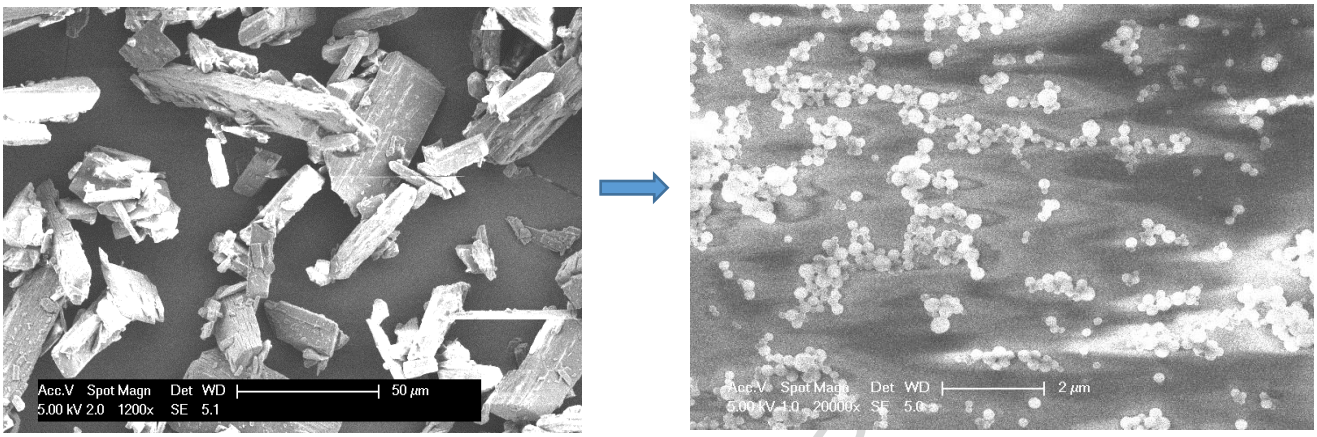
20. Butler JM, Dressman JB. The developability classification system: application of biopharmaceutics concepts to formulation development. Journal of pharmaceutical sciences. 2010;99(12):4940-54.
21. Möschwitzer JP. Drug nanocrystals in the commercial pharmaceutical development process. International Journal of Pharmaceutics. 2013;453(1):142-56.
22. Van Eerdenbrugh B, Van den Mooter G, Augustijns P. Top-down production of drug nanocrystals: Nanosuspension stabilization, miniaturization and transformation into solid products. International Journal of Pharmaceutics. 2008;364(1):64-75.
23. [updated May 29, 2015May 31, 2015]; [Food and drug Administration, Center for Drug Evaluation and Research . Orange book: Office of Generic Drugs]. Available from: http://www.accessdata.fda.gov/scripts/cder/ob/default.cfm
24. Dong Y, Ng WK, Shen S, Kim S, Tan RBH. Controlled antisolvent precipitation of spironolactone nanoparticles by impingement mixing. International Journal of Pharmaceutics. 2011;410(1–2):175-9.
25. Dong Y, Ng WK, Hu J, Shen S, Tan RBH. A continuous and highly effective static mixing process for antisolvent precipitation of nanoparticles of poorly water-soluble drugs. International Journal of Pharmaceutics. 2010;386(1–2):256-61.
26. Shi Y, Porter W, Merdan T, Li LC. Recent advances in intravenous delivery of poorly water-soluble compounds. Expert Opinion on Drug Delivery. 2009;6(12):1261-82.
27. Yadav D, Kumar N. Nanonization of curcumin by antisolvent precipitation: Process development, characterization, freeze drying and stability performance. International Journal of Pharmaceutics. 2014;477(1–2):564-77.
28. Mugheirbi NA, Paluch KJ, Tajber L. Heat induced evaporative antisolvent nanoprecipitation (HIEAN) of itraconazole. International Journal of Pharmaceutics. 2014;471(1–2):400-11.
29. Thorat AA, Dalvi SV. Liquid antisolvent precipitation and stabilization of nanoparticles of poorly water soluble drugs in aqueous suspensions: Recent developments and future perspective. Chemical Engineering Journal. 2012;181–182(0):1-34.
30. Zhang H-X, Wang J-X, Zhang Z-B, Le Y, Shen Z-G, Chen J-F. Micronization of atorvastatin calcium by antisolvent precipitation process. International Journal of Pharmaceutics. 2009;374(1–2):106-13.
31. Zu Y, Wu W, Zhao X, Li Y, Wang W, Zhong C, Zhang Y, Zhao X. Enhancement of solubility, antioxidant ability and bioavailability of taxifolin nanoparticles by liquid antisolvent precipitation technique. International Journal of Pharmaceutics. 2014;471(1–2):366-76.
32. Zu Y, Sun W, Zhao X, Wang W, Li Y, Ge Y, Liu Y, Wang K. Preparation and characterization of amorphous amphotericin B nanoparticles for oral administration through liquid antisolvent precipitation. European Journal of Pharmaceutical Sciences. 2014;53(0):109-17.
33. Zhao H, Wang J, Zhang H, Shen Z, Yun J, Chen J. Facile Preparation of Danazol Nanoparticles by High-Gravity Anti-solvent Precipitation (HGAP) Method. Chinese Journal of Chemical Engineering. 2009;17(2):318-23.
34. Dong Y, Ng WK, Shen S, Kim S, Tan RBH. Preparation and characterization of spironolactone nanoparticles by antisolvent precipitation. International Journal of Pharmaceutics. 2009;375(1–2):84-8.
35. Douroumis D, Scheler S, Fahr A. Using a modified shepards method for optimization of a nanoparticulate cyclosporine a formulation prepared by a static mixer technique. Journal of pharmaceutical sciences. 2008;97(2):919-30.
36. Douroumis D, Fahr A. Nano- and micro-particulate formulations of poorly water-soluble drugs by using a novel optimized technique. European Journal of Pharmaceutics and Biopharmaceutics. 2006;63(2):173-5.
37. Thakur R, Vial C, Nigam K, Nauman E, Djelveh G. Static mixers in the process industries—a review. Chemical Engineering Research and Design. 2003;81(7):787-826.

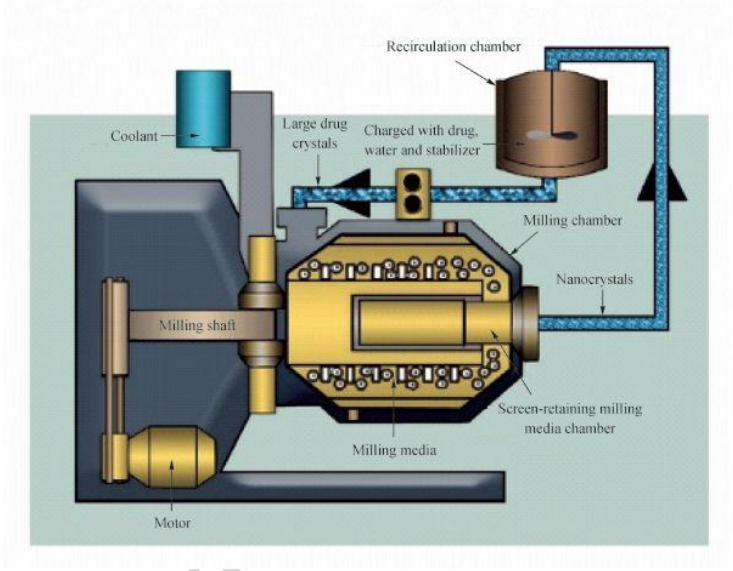
38. Hu J, Ng WK, Dong Y, Shen S, Tan RBH. Continuous and scalable process for water-redispersible nanoformulation of poorly aqueous soluble APIs by antisolvent precipitation and spray-drying. International Journal of Pharmaceutics. 2011;404(1–2):198-204.
39. Nanjwadea BK, Derkara GK, Bechraa H, Manvib F. Nanosized technological approaches for the delivery of poorly water soluble drugs. Iranian Journal of Pharmaceutical Sciences. 2010;6(3):149-62.
40. Dhumal RS, Biradar SV, Yamamura S, Paradkar AR, York P. Preparation of amorphous cefuroxime axetil nanoparticles by sonoprecipitation for enhancement of bioavailability. European Journal of Pharmaceutics and Biopharmaceutics. 2008;70(1):109-15.
41. Chan H-K, Kwok PCL. Production methods for nanodrug particles using the bottom-up approach. Advanced Drug Delivery Reviews. 2011;63(6):406-16.
42. Chen J-F, Zhou M-Y, Shao L, Wang Y-Y, Yun J, Chew NYK, Yun J, Chew N, Chan H. Feasibility of preparing nanodrugs by high-gravity reactive precipitation. International Journal of Pharmaceutics. 2004;269(1):267-74.
43. Shao L, Chen J. Synthesis and application of nanoparticles by a high gravity method. China Particuology. 2005;3(1–2):134-5.
44. Zhong J, Shen Z, Yang Y, Chen J. Preparation and characterization of uniform nanosized cephradine by combination of reactive precipitation and liquid anti-solvent precipitation under high gravity environment. International Journal of Pharmaceutics. 2005;301(1–2):286-93.
45. Dhumal RS, Biradar SV, Paradkar AR, York P. Particle engineering using sonocrystallization: Salbutamol sulphate for pulmonary delivery. International Journal of Pharmaceutics. 2009;368(1–2):129-37.
46. Chen J-F, Zhang J-Y, Shen Z-G, Zhong J, Yun J. Preparation and characterization of amorphous cefuroxime axetil drug nanoparticles with novel technology: high-gravity antisolvent precipitation. Industrial & engineering chemistry research. 2006;45(25):8723-7.
47. Campardelli R, Baldino L, Reverchon E. Supercritical fluids applications in nanomedicine. The Journal of Supercritical Fluids. 2015;101(0):193-214.
48. Keshavarz A, Karimi-Sabet J, Fattahi A, Golzary A, Rafiee-Tehrani M, Dorkoosh FA. Preparation and characterization of raloxifene nanoparticles using Rapid Expansion of Supercritical Solution (RESS). The Journal of Supercritical Fluids. 2012;63(0):169-79.
49. Keshmiri K, Vatanara A, Tavakoli O, Manafi N. Production of ultrafine clobetasol propionate via rapid expansion of supercritical solution (RESS): Full factorial approach. The Journal of Supercritical Fluids. 2015;101(0):176-83.
50. Uchida H, Nishijima M, Sano K, Demoto K, Sakabe J, Shimoyama Y. Production of theophylline nanoparticles using rapid expansion of supercritical solutions with a solid cosolvent (RESS-SC) technique. The Journal of Supercritical Fluids. (0).
51. Samei M, Vatanara A, Fatemi S, Rouholamini Najafabadi A. Process variables in the formation of nanoparticles of megestrol acetate through rapid expansion of supercritical CO2. The Journal of Supercritical Fluids. 2012;70(0):1-7.
52. Sane A, Limtrakul J. Formation of retinyl palmitate-loaded poly(l-lactide) nanoparticles using rapid expansion of supercritical solutions into liquid solvents (RESOLV). The Journal of Supercritical Fluids. 2009;51(2):230-7.
53. Türk M, Bolten D. Formation of submicron poorly water-soluble drugs by rapid expansion of supercritical solution (RESS): Results for Naproxen. The Journal of Supercritical Fluids. 2010;55(2):778-85.
54. Thakur R, Gupta RB. Formation of phenytoin nanoparticles using rapid expansion of supercritical solution with solid cosolvent (RESS-SC) process. International Journal of Pharmaceutics. 2006;308(1–2):190-9.
55. Mezzomo N, Rosso Comim SR, Campos CEM, Ferreira SRS. Nanosizing of sodium ibuprofen by SAS method. Powder Technology. 2015;270, Part A(0):378-86.

56. Esfandiari N, Ghoreishi SM. Synthesis of 5-Fluorouracil nanoparticles via supercritical gas antisolvent process. The Journal of Supercritical Fluids. 2013;84(0):205-10.
57. Kim YH, Shing KS. Supercritical fluid-micronized ipratropium bromide for pulmonary drug delivery. Powder Technology. 2008;182(1):25-32.
58. Tabernero A, Martín del Valle EM, Galán MA. Precipitation of tretinoin and acetaminophen with solution enhanced dispersion by supercritical fluids (SEDS). Role of phase equilibria to optimize particle diameter. Powder Technology. 2012;217(0):177-88.
59. Chattopadhyay P, Gupta RB. Production of griseofulvin nanoparticles using supercritical CO2 antisolvent with enhanced mass transfer. International Journal of Pharmaceutics. 2001;228(1–2):19-31.
60. Bürki K, Jeon I, Arpagaus C, Betz G. New insights into respirable protein powder preparation using a nano spray dryer. International Journal of Pharmaceutics. 2011;408(1–2):248-56.
61. Nano Spray Dryer B-90. Available from: http://www.buchi.com/en/products/spray-drying-and-encapsulation/nano-spray-dryer-b-90
62. Baba K, Nishida K. Calpain inhibitor nanocrystals prepared using Nano Spray Dryer B-90. Nanoscale Res Lett. 2012;7(1):436.
63. Harsha SN, Aldhubiab BE, Nair AB, Alhaider IA, Attimarad M, Venugopala KN, Srinivasan S, Gangadhar N, Asif AH. Nanoparticle formulation by Buchi B-90 Nano Spray Dryer for oral mucoadhesion. Drug Des Devel Ther. 2015;9:273-82.
64. Hu J, Johnston KP, Williams Iii RO. Rapid dissolving high potency danazol powders produced by spray freezing into liquid process. International Journal of Pharmaceutics. 2004;271(1–2):145-54.
65. de Waard H, Grasmeijer N, Hinrichs WLJ, Eissens AC, Pfaffenbach PPF, Frijlink HW. Preparation of drug nanocrystals by controlled crystallization: Application of a 3-way nozzle to prevent premature crystallization for large scale production. European Journal of Pharmaceutical Sciences. 2009;38(3):224-9.
66. Alam MA, Al-Jenoobi FI, Al-mohizea AM. Commercially bioavailable proprietary technologies and their marketed products. Drug Discovery Today. 2013;18(19–20):936-49.
67. Shegokar R, Müller RH. Nanocrystals: Industrially feasible multifunctional formulation technology for poorly soluble actives. International Journal of Pharmaceutics. 2010;399(1–2):129-39.
68. Tzu-Lan CHANG HZ, Danni LIANG, Jun F. LIANG. Nanocrystal technology for drug formulation and delivery. Front Chem Sci Eng. 2015;9(1):1-14.
69. Knieke C, Azad MA, Davé RN, Bilgili E. A study of the physical stability of wet media-milled fenofibrate suspensions using dynamic equilibrium curves. Chemical Engineering Research and Design. 2013;91(7):1245-58.
70. Li M, Yaragudi N, Afolabi A, Dave R, Bilgili E. Sub-100 nm drug particle suspensions prepared via wet milling with low bead contamination through novel process intensification. Chemical Engineering Science. 2015;130(0):207-20.
71. Yao Q, Tao X, Tian B, Tang Y, Shao Y, Kou L, Gou J, Li X, Yin T, Tang X. Improved oral bioavailability of core–shell structured beads by redispersion of the shell-forming nanoparticles: Preparation, characterization and in vivo studies. Colloids and Surfaces B: Biointerfaces. 2014;113(0):92-100.
72. Niwa T, Miura S, Danjo K. Universal wet-milling technique to prepare oral nanosuspension focused on discovery and preclinical animal studies – Development of particle design method. International Journal of Pharmaceutics. 2011;405(1–2):218-27.
73. Cerdeira AM, Mazzotti M, Gander B. Formulation and drying of miconazole and itraconazole nanosuspensions. International Journal of Pharmaceutics. 2013;443(1–2):209-20.
74. Kumar S, Burgess DJ. Wet milling induced physical and chemical instabilities of naproxen nano-crystalline suspensions. International Journal of Pharmaceutics. 2014;466(1–2):223-32.
75. Muller RH, Becker R, Kruss B, Peters K. Pharmaceutical nanosuspensions for medicament administration as systems with increased saturation solubility and rate of solution. Google Patents; 1999.

76. Keck CM, Müller RH. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. European Journal of Pharmaceutics and Biopharmaceutics. 2006;62(1):3-16.
77. Homogenization [updated May, 2015; cited 2015 31 May]. Available from: http://www.substech.com/dokuwiki/doku.php?id=homogenization
78. Radtke M. Pure drug nanoparticles for the formulation of poorly soluble drugs. New Drugs. 2001;3(62-68).
79. Kakran M, Shegokar R, Sahoo NG, Al Shaal L, Li L, Müller RH. Fabrication of quercetin nanocrystals: Comparison of different methods. European Journal of Pharmaceutics and Biopharmaceutics. 2012;80(1):113-21.
80. Anwar M, Warsi MH, Mallick N, Akhter S, Gahoi S, Jain GK, Talegaonkar S, Ahmad FJ, Khar RK. Enhanced bioavailability of nano-sized chitosan–atorvastatin conjugate after oral administration to rats. European Journal of Pharmaceutical Sciences. 2011;44(3):241-9.
81. Xu L-M, Hu T-T, Pu Y, Le Y, Chen J-F, Wang J-X. Preparation of high-performance ultrafine budesonide particles for pulmonary drug delivery. Chemical Engineering Journal. 2014;252(0):281-7.
82. Sun W, Tian W, Zhang Y, He J, Mao S, Fang L. Effect of novel stabilizers—cationic polymers on the particle size and physical stability of poorly soluble drug nanocrystals. Nanomedicine: Nanotechnology, Biology and Medicine. 2012;8(4):460-7.
83. Guo J-j, Yue P-F, Lv J-l, Han J, Fu S-s, Jin S-x, Jin SY, Yuan HL. Development and in vivo/in vitro evaluation of novel herpetrione nanosuspension. International Journal of Pharmaceutics. 2013;441(1–2):227-33.
84. Xiong R, Lu W, Li J, Wang P, Xu R, Chen T. Preparation and characterization of intravenously injectable nimodipine nanosuspension. International Journal of Pharmaceutics. 2008;350(1–2):338-43.
85. Salazar J. Combinative particle size reduction technologies for the formulation of poorly soluble drugs: Freie Universität Berlin, Germany; 2013.
86. Xu L-M, Zhang Q-X, Zhou Y, Zhao H, Wang J-X, Chen J-F. Engineering drug ultrafine particles of beclomethasone dipropionate for dry powder inhalation. International Journal of Pharmaceutics. 2012;436(1–2):1-9.
87. Salazar J, Müller RH, Möschwitzer JP. Application of the combinative particle size reduction technology H 42 to produce fast dissolving glibenclamide tablets. European Journal of Pharmaceutical Sciences. 2013;49(4):565-77.
88. Al Shaal L, Shegokar R, Müller RH. Production and characterization of antioxidant apigenin nanocrystals as a novel UV skin protective formulation. International Journal of Pharmaceutics. 2011;420(1):133-40.
89. Lee J, Lee S-J, Choi J-Y, Yoo JY, Ahn C-H. Amphiphilic amino acid copolymers as stabilizers for the preparation of nanocrystal dispersion. European Journal of Pharmaceutical Sciences. 2005;24(5):441-9.
90. Bose S, Schenck D, Ghosh I, Hollywood A, Maulit E, Ruegger C. Application of spray granulation for conversion of a nanosuspension into a dry powder form. European Journal of Pharmaceutical Sciences. 2012;47(1):35-43.
91. Ain-Ai A, Gupta PK. Effect of arginine hydrochloride and hydroxypropyl cellulose as stabilizers on the physical stability of high drug loading nanosuspensions of a poorly soluble compound. International Journal of Pharmaceutics. 2008;351(1–2):282-8.
92. Yue P-F, Wan J, Wang Y, Li Y, Ma Y-Q, Yang M, Hu PY, Yuan HL, Wang CH. d-Alpha-tocopherol acid polyethylene glycol 1000 succinate, an effective stabilizer during solidification transformation of baicalin nanosuspensions. International Journal of Pharmaceutics. 2013;443(1–2):279-87.
93. Quan P, Shi K, Piao H, Piao H, Liang N, Xia D, Cui F. A novel surface modified nitrendipine nanocrystals with enhancement of bioavailability and stability. International Journal of Pharmaceutics. 2012;430(1–2):366-71.
94. Van Eerdenbrugh B, Froyen L, Van Humbeeck J, Martens JA, Augustijns P, Van Den Mooter G. Alternative matrix formers for nanosuspension solidification: Dissolution performance and X-ray

microanalysis as an evaluation tool for powder dispersion. European Journal of Pharmaceutical Sciences. 2008;35(4):344-53.
95. Lai F, Pini E, Corrias F, Perricci J, Manconi M, Fadda AM, Sinico C. Formulation strategy and evaluation of nanocrystal piroxicam orally disintegrating tablets manufacturing by freeze-drying. International Journal of Pharmaceutics. 2014;467(1–2):27-33.
96. Ohshima H, Miyagishima A, Kurita T, Makino Y, Iwao Y, Sonobe T, Itai S. Freeze-dried nifedipine-lipid nanoparticles with long-term nano-dispersion stability after reconstitution. International Journal of Pharmaceutics. 2009;377(1–2):180-4.
97. Parsian AR, Vatanara A, Rahmati MR, Gilani K, Khosravi KM, Najafabadi AR. Inhalable budesonide porous microparticles tailored by spray freeze drying technique. Powder Technology. 2014;260(0):36-41.





Nanosizing by Media milling technique

Graphical abstract