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Review Article

Recent Trends in Top-Down Technologies for the Preparation of Nanosuspensions

Ravinder Kodipyaka[1], Sumalatha Govindu[2,*]

[1]Department of Pharmacy, Chaitanya (Deemed to be University), Kishanpura, Hanumakonda, Warangal, Telangana, INDIA.
[2]Department of Pharmacognosy, Sri Venkateswara College of Pharmacy, Chittoor, Andhra Pradesh, INDIA.

ABSTRACT

The increasing demands for drugs and advancements in drug discovery have led to the development of numerous potent molecules with unfavourable solubility and bioavailability characteristics. These challenges are driving significant developments in formulation technologies, such as nanosuspensions, which can potentially address these challenges and improve the success rate of new molecules. Nanosuspensions have unique properties due to their size, which can enhance solubility, adapt to surface modification and mucoadhesion for drug targeting. However, key aspects to consider when designing nanosuspensions include stability in liquid and solid states and dispersibility without aggregation. Multiple techniques and formulation approaches are employed to design and develop nanosuspension formulations with desired characteristics. Top-down methods such as media milling and high-pressure homogenization have drawn interest from researchers in both academia and the pharmaceutical industry due to their high level of adoptability and scalability. As a result, numerous innovative oral and parenteral formulations are being developed, reaching patients globally and serving their unmet needs.

Keywords: Nanosuspension, Solubility and bioavailability enhancement, Preparation methods, Formulation considerations, Characterization techniques.

Correspondence:
Dr. Sumalatha Govindu
Professor and Head, Department of Pharmacognosy, Sri Venkateswara College of Pharmacy, Chittoor, Andhra Pradesh, INDIA.
Email: sumalatha2k@gmail.com

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INTRODUCTION

Modern technologies have significantly advanced the creation of numerous new molecules with high efficacy. However, solubility and bioavailability are major hindrances to the successful development of new pharmaceutical products, especially for drugs belonging to BCS class II and IV.[1-5]

Conventional approaches to improve solubility and bioavailability include micronization, cosolvents, surfactants, solubilizers, pH adjustment methods and other techniques. However, these conventional solubility enhancement approaches have limited applicability. Usage of micronization equipment can enhance solubility to a limited extent as a result of decrease in particle size and increase in surface area.[6-8] Also, conventional approaches have been shown to be less effective for drugs like 'grease balls' which exhibit high lipophilicity (logP) and poor aqueous solubility and 'brick dust' molecules which exhibit high melting point, low lipophilicity and poor aqueous solubility due to strong inter molecular bonding and high lattice energy in solid state.[9]

While other delivery methods such as liposomal technology, crystalline and amorphous solid dispersion technologies, complexation techniques[10-13] have been used to address solubility and bioavailability related challenges, application of these technologies is highly dependent not only on nature and properties of the drug molecule under consideration but also the choice of solvent or solvent mixtures thus limiting their applicability in formulation development.

Nanosuspensions have emerged as a potential alternative for improving solubility with significant advantages including high drug loading feasibility thus allowing high dose administration, minimal restrictions related to solvent selection, suitability for conversion into a solid state for enhanced stability and application flexibility for various routes of administration.

Nanosuspensions are submicron sized particulate dispersions in aqueous or non-aqueous vehicles, stabilised by surface active agents and polymeric materials[9] with typical size range of 1-100 nm, though it can used for the particles of larger diameter.[14] The various techniques used to achieve nano size range of drug particles can be broadly classified under three categories: 1) Top-down techniques 2) Bottom-up techniques 3) Combinative technologies.[15,16]

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Nanosuspensions carry significant advantages in formulation technology space due to reduced particle size, high saturation solubility, enhanced dissolution and improved bioavailability. This technology is suitable for both 'brick dust' and 'grease ball' molecules,[4-9] and opens up possibilities for dose reduction and enhanced safety owing to improvement in biopharmaceutical profile and biological performance of the drug molecules.

Decrease in particle size to nano range with resultant increase in surface area, saturation solubility and dissolution velocity is well described through Nernst-Brunner, Noyes-Whitney and Ostwald-Freundlich equations.

Nanosuspensions also carry certain limitations due to challenges associated with physical stability, sedimentation issues and particle growth, which can lead to product quality and *in vivo* efficacy issues upon long-term storage.

METHODS OF PREPARATION

As briefed in previous section, various methods used for the preparation of nanosuspensions fall under three main categories viz., Top-down, Bottom-up and Combinative technologies as summarised in Figure 1. As the name indicates, Top-down methods start with larger particles and break them down to form nanosized particles whereas Bottom-up methods generally start with molecular form of the drug and progressively grow into larger particles. The subsequent sections of this review provide elaborate description of top-down methods which have proven to be useful not only for research but also for industrial applications.

High pressure homogenization

In this technique, the product is exposed to high pressure zone by pumping it through a restricted space designed within the homogenizer. High pressure homogenizers used in homogenization can be classified into two main categories based on the design of homogenization chamber and fluid flow: 1) Piston-gap homogenizers and 2) Jet-stream homogenizers or microfluidizers.

Piston-gap homogenizers-Homogenization in aqueous media (DissoCubes)

Piston-gap homogenizers commonly used for homogenization in aqueous media are commercially available from APV Gaulin, Avestin, Stansted fluid power and Niro Soavi. DissoCubes is a technology used for homogenization in aqueous media using Piston-gap homogenizer for particle size reduction and was originally developed by Muller *et al.*, in the year 1994 and later acquired by Skye Pharma.

The Piston-gap homogenizer assembly consists of a high-pressure pump, valve assembly and impact ring. High-pressure pump regulates the pressure of the product or premix passing through the narrow space. The valve assembly, a critical component, impacts size reduction and droplet disruption during homogenization. There are three different types of valves that vary in design and geometry: radial diffuser valve, counter jet valve and axial flow through orifice value. The radial diffuser valve is commonly used component in high pressure homogenization, forming a narrow gap between the rod and seat. This results in particle disruption due to the intense increase in fluid velocity and decrease in pressure.[17] After passing through the high impact zone, the fluid hits the surface of the impact ring followed by deflection and exit from homogenizer (Figure 2).

The laboratory scale Piston-gap homogenizer typically consists of 40 mL capacity and can operate at a pressure of 100 to 1500 bars. The pre-suspension is prepared using a high-speed stirrer and passed through piston-gap in multiple cycles typically 5-20 cycles at 1000-1500 bar pressure. The decrease in diameter from 3 cm to 3-25 µm (in a laboratory homogenizer) results in increased dynamic pressure and decreased static pressure below the boiling point of water at room temperature. This results in the formation of gas bubbles, which implode when the suspension leaves the zone, forming cavitation and shear forces that break larger particles.[15] Cavitation is the main effect contributing to particle break-down in Piston-gap homogenizers. The cavitation effect can be more pronounced if dispersion medium exhibits high vapor pressure at room temperature.

Requirements such as the need for pre-processing to get the input material in micronized size range,[18] and necessity for expensive equipment are main disadvantages of using this technique. Apart from the cost, the technique has limited use for water-sensitive and thermolabile drugs and drugs having low melting point.[19]

Celecoxib nanosuspensions formulated using TPGS (tocopheryl polyethylene glycol succinate) as a surfactant stabilizer and processed using high pressure homogenization followed by freeze drying displayed a higher dissolution (90.8% in 60 min) and significant improvement in Cmax and $AUC_{(0-t)}$ of nanosuspension compared to Celecoxib standard powder.[20]

Jin Xie *et al.*, have prepared surfactant-free nanosuspensions using co-processed nanosuspensions of Nanocrystalline Cellulose-Sodium Carboxymethyl Starch (NCCS) as synergistic stabilizer and Baicalin as model drug by using piston-gap high pressure homogenizer. These Baicalin nanosuspensions exhibited a fast dissolution rate and twice the AUC (0-∞) when compared with crude Baicalin.[21]

Curcumin nanosuspensions with surface adsorption of surfactants such as Tocopheryl Polyethylene Glycol Succinate (TPGS) and polysorbate 80 have exhibited higher levels of drug in rats compared to TPGS coated nanosuspensions and curcumin solution.[22]

Mohsen Hedaya *et al.*, prepared Ibuprofen oral nanosuspensions using polyvinylpyrrolidone K30 as a stabilizer and Tween

80 as a surfactant. They conducted pharmacokinetic studies comparing them to unhomogenized suspension, nanoparticles, untreated suspension and the marketed product Sapofen® Junior paediatric oral suspension and IV hydroalcoholic solution. The nanosuspension formulation showed a 2-fold increase in bioavailability.[23]

Piston gap homogenizers-Homogenization in Nonaqueous and aqueous mixture (Nanopure®)

Nanopure® is the proprietary technique developed and owned by PharmaSol GmbH[24] uses non-aqueous or low water content media in order to achieve particle size reduction in nano range.

This technology is also known as 'deep-freeze homogenization' where the drug suspension in nonaqueous or low water content medium is homogenized at 0°C or sometimes below the freezing point. It was found that the homogenization can be performed in a nonaqueous media or in aqueous mixture even with nonaqueous media having lower vapour pressure than water. The low vapour pressure of nonaqueous media or water liquid mixtures cause insufficient drop in static pressure to initiate cavitation, the impact of cavitation in the homogenization gap is either completely eliminated or significantly reduced when low temperatures are used for the homogenization process.[25]

One of the distinct advantages with Nanopure® technology is that it is particularly suitable for thermolabile and water sensitive products because of usage of nonaqueous or reduced water content and low processing temperature conditions.

Homogenization using Microfluidizer® Technology

Microfluidizer® technology (Microfluidics Inc, USA) is a technology that works on jet-stream principle. In this process, a liquid stream is passed through micro-channels of an interaction chamber at high pressure (500-30000 psi) to achieve size reduction as a result of high impact, shear forces and high turbulence within the micro-channels of the interaction chamber towards an impingement area where the fluids flow and interact (Figure 3A). The sample is first poured into the inlet reservoir. The constant high-pressure intensifier pump forces the liquid through a fixed geometry Interaction Chamber™ at high pressure ranging from 500 psi to 30000 psi. Finally, the heat removal is done by passing the liquid a heat exchanger followed by sample collection.

The design and construction of interaction chamber is critical for achieving desired globule or particle size. The outer part of it is generally constructed using stainless steel and internal part is using diamond or ceramic materials.

Two different designs of interaction chambers available depending on nature of application.[26,27]

Y-type interaction chamber

In this chamber (Figure 3B), the liquid is divided into 2 streams at the inlet chamber and fluid velocity increases because of decrease in diameter of microchannels. The two liquid streams then collide with one another from two opposite microchannels, resulting in high turbulence, shear and impact forces leading to size reduction of globules or particles. It is primarily used for emulsions, liposomes and polymer encapsulations.

Z-type interaction chamber

In this chamber, a high-pressure liquid stream is forced through microchannels (Figure 3C) which causes particle size reduction due to particle collision, impact with chamber walls and the generation of shear forces. It is primarily used for cell disruption, deagglomeration, particle size reduction and dispersions.

Single slotted chambers are generally employed for small batches in a laboratory set up whereas multi-slotted chambers designed with parallel micro channels (Figures 3D and 3E) are used in large volume production.

Skyepharma developed Fenofibrate drug product using IDD®-P technology platform for the treatment of elevated blood lipid disorders. This product was successfully commercialized in USA market in 2005 under the brand name of TRIGLIDE®.[28]

Cyclosporine A nanosuspensions prepared using the microfludization method have shown that the combination of drug, hydroxypropyl methyl cellulose polymer and Soluplus® in a 1:1:0.5 w/w ratio was giving optimum stabilization with about a 2-fold increase in solubility in comparison with the coarse Cyclosporine A formulation.[29]

Alptug Karakucuk *et al.*, prepared Ritonavir nanosuspensions by microfluidization using DoE studies. They found 3.5-fold increase in solubility when compared with coarse powder. Also, significant improvement in biorelevant dissolution media observed compared to commercial Norvir® product.[30]

Sudhir Verma *et al.*, employed QbD principles, used Indomethacin as model drug and established critical formulation and process variables by using microfluidization method.[31]

Bexarotene nanocrystals, prepared using microfluidization, showed significant improvement in dissolution rate and bioavailability when surface was modified with folate-chitosan conjugation. The nanocrystals' surface showed low polydispersity index with a stable zeta potential of ~24.6 mV.[32]

Shweta Sharma *et al.*, created Paclitaxel nanocrystals using microfluidization and lyophilization. They found polysorbate 80 and low molecular weight Poly styrene sulfonates are effective in reducing particle size and stabilizing nanosuspensions. Further studies have shown that the prepared Paclitaxel nanocrystals were more potent with better efficacy compared to Paclitaxel solution.

Oral pharmacokinetic studies in rats showed a significant increase in bioavailability, indicating potential use for oral chemotherapy.[33]

Budesonide nanosuspension prepared using microfluidizer demonstrated high pulmonary delivery and distribution than standard particles.[34]

Media Milling

Media milling is often known as wet milling because of usage of aqueous system for the operation. Media milling of formulation mixture consisting of drug and suitable excipients is carried out using agitator bead mills with the help of a high-speed rotating agitator (typically 20000 rotations per minute) in a milling chamber in presence of grinding or milling media. The grinding balls sized 0.03-30 mm create mechanical stress and impact forces with resultant decrease the size of feed material. The final material is collected from the outlet after separating it from griding media.[35]

After the collection of final material from the outlet, the liquid slurry is subjected to degassing or drying, depending on its end use. If the desired final product is a liquid dispersion, degassing is done to ensure the release of air or gases entrapped in the suspension medium. An additional drying step is employed if the desired final product is a powder, often using a fluidized bed or flash drying equipment. Dry powders can be conveniently converted into solid dosage forms like tablets or capsules. Similarly, sterile wet milling process is used to produce formulations for parenteral administration.

Elan Drug Delivery has acquired patented NanoCrystal® technology from Nano Systems and developed an immunosuppressant solid oral formulation of Sirolimus (Rapamune® tablets), which was the first product approved drug product by the USFDA.[36]

In recent years, usage of milling media of smaller diameter of less than 100 mm, to bring about the nanonization of drug particles. In order to achieve effective separation of such small size grinding media, Kotobuki has introduced Ultra Apex Mill that utilizes centrifugation principle to separate even a small media of 15 mm in size.[35]

The media milling is one of widely used technique by pharmaceutical industry for producing nanocrystal-based formulations, predominantly in injectable and oral space. While several formulations are now on the market,[35,37] numerous other formulations are at different stages of drug development.

Laser Ablation and Fragmentation method

Laser ablation and fragmentation are the well-known methods extensively used for producing inorganic nanomaterials. In recent years, laser ablation techniques have emerged as novel techniques and gained popularity in pharmaceutical applications. These techniques carry few distinctive advantages over others because of their simplicity, speed, reproducibility, chemical-free and environmentally friendly processes.

Laser ablation is typically carried out using a laser beam intensity to ablate or remove material generally from a solid surface in a liquid medium to produce nanoparticles. Though both Nanosecond and Femtosecond methods are available, the Femtosecond method is reported to be a preferred option because of the application of ultrashort laser pulses resulting in minimal degradation of the active material.[38] On the other hand, laser fragmentation is either

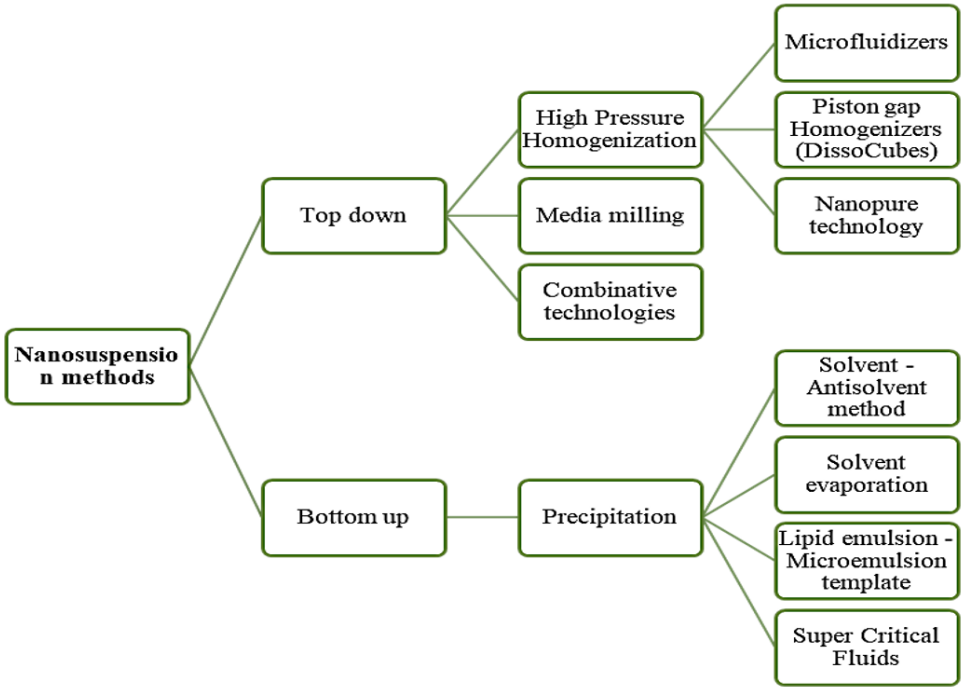

Figure 1: Major categories of preparation techniques employed for nanosuspensions.

a follow-up step to control the particle size or as a distinct process in which laser beam is applied to break down bigger particles in a liquid dispersion under continuous mixing.[39]

Weimeng Ding *et al*., efficiently produced Paclitaxel nanocrystals, but observed an increase in degradation and drug hydration.[40] Studies using Megestrol acetate,[41] Beclomethasone dipropionate,[42] Fenofibrate and Naproxen ingredients[43] showed general trends related to the particle size and drug degradation, improved dissolution profile.

Pulsed laser ablation method was applied successfully to achieve particle size reduction of selected anti-inflammatory drugs such as Meloxicam, Ibuprofen and Niflumic acid through careful selection of laser parameters.[44]

Laser ablation technique was used to prepare nanosized Curcumin, Ritonavir and Atazanavir in presence of Pluronic® F127 in aqueous environment. These nanoformulations displayed improved water dispersibility, ultrasmall size, enhanced cellular uptake, improved drug delivery across blood-brain barrier and achieved significant reduction in viral marker levels. The laser-ablated nanoparticles showed low toxicity and enhanced cellular uptake during cell line studies.[45]

Combinative technologies

Combinative technologies are methods that combine multiple techniques to reduce particle size efficiently and effectively. They address challenges like the need for micronized material and multiple cycle treatment, which can lead to long processing times and limited drug delivery systems. These technologies aim to improve productivity, cost efficiency and product performance by introducing a pre-treatment step to modify starting material properties before high energy processing. Baxter's Nanoedge®[46,47] and Abbott's SmartCrystal®[48] are two existing combinative technology platforms. Currently, there are five combinative technologies available for producing nano-sized particles: NANOEDGE, H69, H42, H96 and CT combination technology.[49]

Nanoedge® Technology

Baxter has developed a combination technology for creating nanoparticle-based injectable formulations. This involves a solvent-antisolvent technique combined with a top-down method such as high-pressure homogenization. In this process, the drug is dissolved in an organic solvent first followed by mixing with an aqueous medium, then subjecting the resulting pre-suspension containing drug precipitate to a top-down process to achieve nanonization. This process converts thermodynamically unstable drug particles into more stable states by applying single or multiple energy cycles followed by thermal relaxation. The main challenge in this technique is the difficulty in removing organic solvent residues, making large-scale production more complicated and expensive for injectable formulation manufacturing in strict sterile environments.

H69 SmartCrystal Technology

The H69 process is one of the second-generation technologies covered under SmartCrystal® platform technology. It combines microprecipitation and high-pressure homogenization, allowing drug precipitation and particle formation in a high-energy zone of the high-pressure homogenizer. The drug particles thus formed are immediately subjected to cavitation, shear forces and particle collision. Hence, this process is also known as 'cavi-precipitation'. Because the drug particles undergo high energy annealing just after precipitation, this method has the benefit of being able to stop crystal nucleation and particle growth and produce stable crystalline form. A drawback of this process is the difficulty in removal of organic solvent residues as seen in Nanoedge® technology.

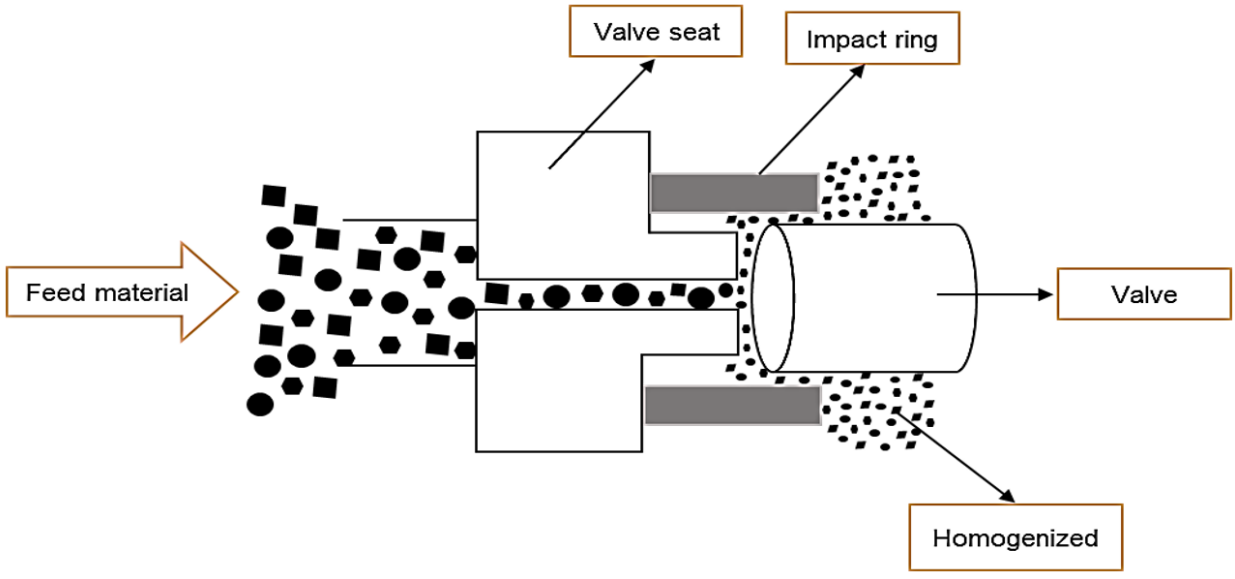

Figure 2: Schematic diagram of Piston-gap Homogenizer.

H42 SmartCrystal Technology

This technology is also one of the variants of different technologies combined under SmartCrystal® technology. This technology is a combination of Spray Drying (SD) as a pre-treatment step followed by high-pressure homogenization for achieving particle size. In first step, drug is dissolved in an organic solvent and subjected to spray drying operation. The spray dried powder obtained in bottom-up stage is redispersed in aqueous system along with surfactants for the stabilization of particles. The aqueous dispersion is subjected to high-pressure homogenization in order to obtain desired nanosuspension. The major advantage of this technique over Nanoedge® and H69 is that the organic solvent is eliminated in first step i.e., bottom-up stage to get solvent-free dry material with reduced number of cycles of homogenization. However, the need for high temperature conditions during spray drying step could become a limiting factor for thermosensitive molecules.

H96 SmartCrystal Technology

Similar to H69 and H42, this technology also belongs to SmartCrystal® family of technologies. In this technology, Lyophilization or Freeze-Drying (FD) is used as a pre-treatment step and resultant material is subjected to high pressure homogenization for getting desired particle size distribution. One of the main advantages with this technology is that the organic solvent is effectively removed during freeze-drying stage making nanosuspension ready for next stage of production process. Although, lyophilization is time intensive process, this technology is especially suitable for expensive and thermolabile molecules because of high yields and low temperature conditions employed during process.

Combination Technology (CT)

This technology differs from other combinative technologies in that it employs two top-down methods for achieving particle size reduction. This technology was developed by Petersen using low-energy pearl mill and high-pressure homogenization. In this process, the combination of shear forces and particle collision with the cavitation effect results in effective particle size reduction.[50]

Rabinow *et al.*, prepared Itraconazole nanosuspensions using the combinative methods consisting of microcrystallization followed by high pressure homogenization and found that intravenous nanosuspension exhibited better pharmacokinetics when compared with solution formulation. The intravenous nanosuspension was well tolerated at high doses and shown enhanced efficacy in rats.[51]

Dnyanesh B. Shelar *et al.*, applied combination of anti-solvent microprecipitation and high pressure homogenization and developed nanosuspension of Isradipine with a particle size of 538 nm. This formulation exhibited enhanced dissolution and improved bioavailability when studied in rats.[52]

Jaime Salazar *et al.*, successfully applied combinative methods of H42 and H96 to the drug Glibenclamide and produced smaller nanoparticles with narrow size distribution.[53]

Zenab Attari *et al.*, prepared nanoparticles of aprepitant and ibuprofen using combination method of ball milling and high pressure homogenization and achieved a particle size of 100 nm.

Table 1: Top-down methods and general applications.

Sl. No.	Top-down technique	Applications
1	Piston-gap homogenizer (DissoCube)	Piston-gap homogenizers commonly used for homogenization in aqueous media. This method works better for thermally stable and high molecular weight compounds.
2	Piston-gap homogenizer (Nanopure)	This method utilizes non-aqueous or low water content media hence particularly suitable for thermolabile and water sensitive products.
3	Jet-stream homogenization (Microfluidizer)	Used for wide range of applications including preparation of emulsions, liposomes and polymer encapsulations using Y-type of interaction chambers; and cell disruption, deagglomeration, particle size reduction and dispersions use Z-type of interaction chambers.
4	Media milling	Widely used technique by pharmaceutical industry for producing a range of nanocrystal-based dosage forms including injections, oral solids and liquids.
5	Laser ablation and Fragmentation	It is not widely used in pharmaceutical industry; However, this technique can be useful in establishing chemical-free and environmentally friendly processes.
6	Combinative technologies	These technologies are highly suitable for molecules that need multiple cycle treatment with prolonged processing times. H69 technology is more suitable for producing stable crystalline forms. H42 is and H96 are appropriate for effective removal of organic solvents from the product. CT methods can be used where effective particle size reduction is necessary.

These formulations showed enhanced dissolution and solubility when compared with unprocessed drug particles.[54]

A brief summary of application and suitability of different top-down methods described above are tabulated below (Table 1).

In addition to Nanoedge® and SmartCrystal® technologies, there are other combination methods used by researchers for the preparation of nanosuspensions. These methods include but not limited to 1) Precipitation-Lyophilization-Homogenization (PLH),[55] Ultrasonic-High Pressure Homogenization (HPH),[56] combination of Rotary evaporation and high-pressure homogenization,[57,58] Melt quench and high-pressure homogenization[57] and Anti-solvent and Ultrasound methods.[59-63]

Formulation Considerations

Nanosuspension formulation carries its significance in drug delivery mechanism because of the unique properties associated with nanoparticles. Nanoparticles possess high surface energy, which can lead to aggregation and destabilization. To address this, various stabilizers are employed and categorized into three types: ionic, non-ionic and polymeric.

Ionic stabilizers such as sodium dodecyl sulfate, docusate sodium work by introducing adsorbed ionic charges, creating mutual repulsion between particles whereas non-ionic stabilizers, like poloxamers and polysorbates act by providing steric stabilization i.e., forming mechanical barriers to prevent particle aggregation.[64] A common strategy for improved stability involves combining ionic and steric stabilizers.

Additionally, for safety reasons, ICH class 3 organic solvents like ethanol and isopropanol are preferred over others when preparing nanosuspensions.[7]

Based on drug nature and dosage form, nanosuspensions may also contain additives like buffers, flavours and cryoprotectants. The selection of stabilizer type and quantity will have significant impact on both the physicochemical stability of the nanosuspension and its behaviour *in vivo*, making these considerations crucial for effective drug delivery systems.

Characterisation Techniques

Solid state characterization

Solid state properties such as crystal form, polymorphism and degree of crystallinity can influence the physical stability including solubility and dissolution of nanosuspensions. In general, thermodynamically most stable crystalline form is preferred over others to minimize or avoid polymorphic transitions during processing, storage and administration of nanosuspensions. High pressure homogenization is a high energy method which may cause crystalline changes including formation of amorphous and metastable polymorphic forms.[65] Hence, it is important to avoid unwanted polymorphic transitions for ensuring long term stability of the system.

The study of solid-state transitions in a drug can be conducted using various characterization techniques such as X-ray powder Diffraction (XRD), Differential Scanning Calorimetry (DSC), thermogravimetry, infrared and Raman spectroscopy studies.

Particle Size distribution

Particle size distribution of the active ingredient is an important characteristic that can influence saturation solubility, dissolution, physicochemical stability. The common methods employed for determining particle size distribution are Laser Diffraction (LD), Dynamic Light Scattering (DLS) also known as Photon Correlation Spectroscopy (PCS) and Coulter Counter.

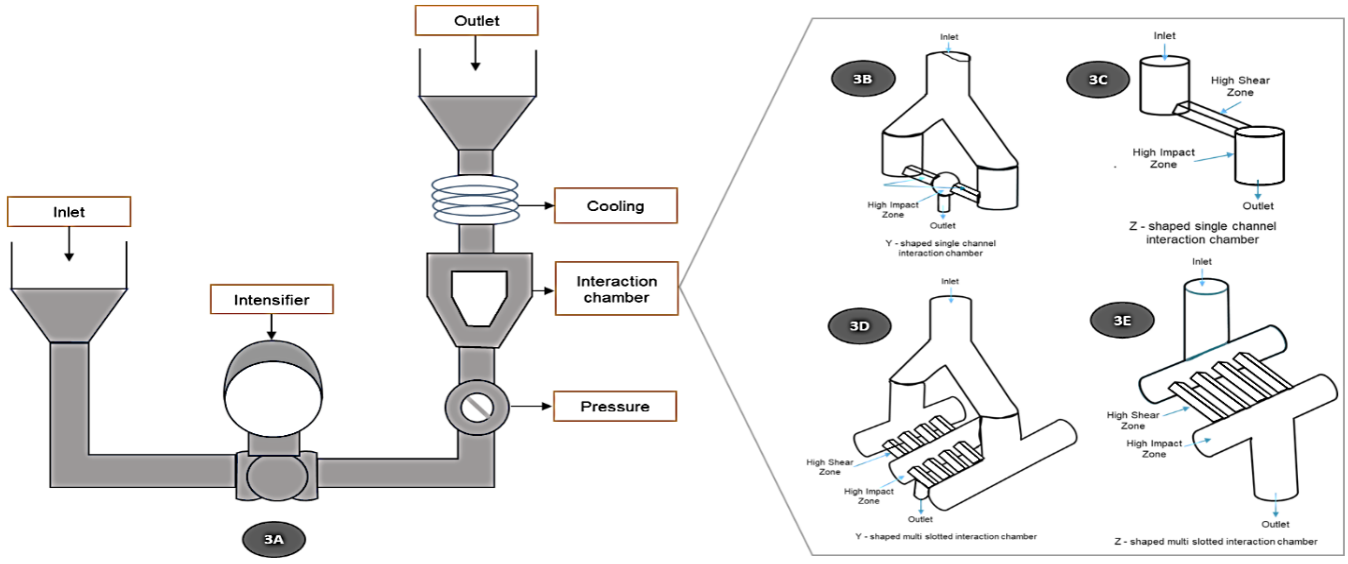

Figure 3: Illustration of high-pressure homogenization using Microfluidizer® technology (3A- Microfluidizer; 3B-'Y' shaped chamber; 3C-'Z' shaped chamber; 3D-'Y' shaped multi slotted chamber; 3E- 'Z' shaped multi slotted chamber).

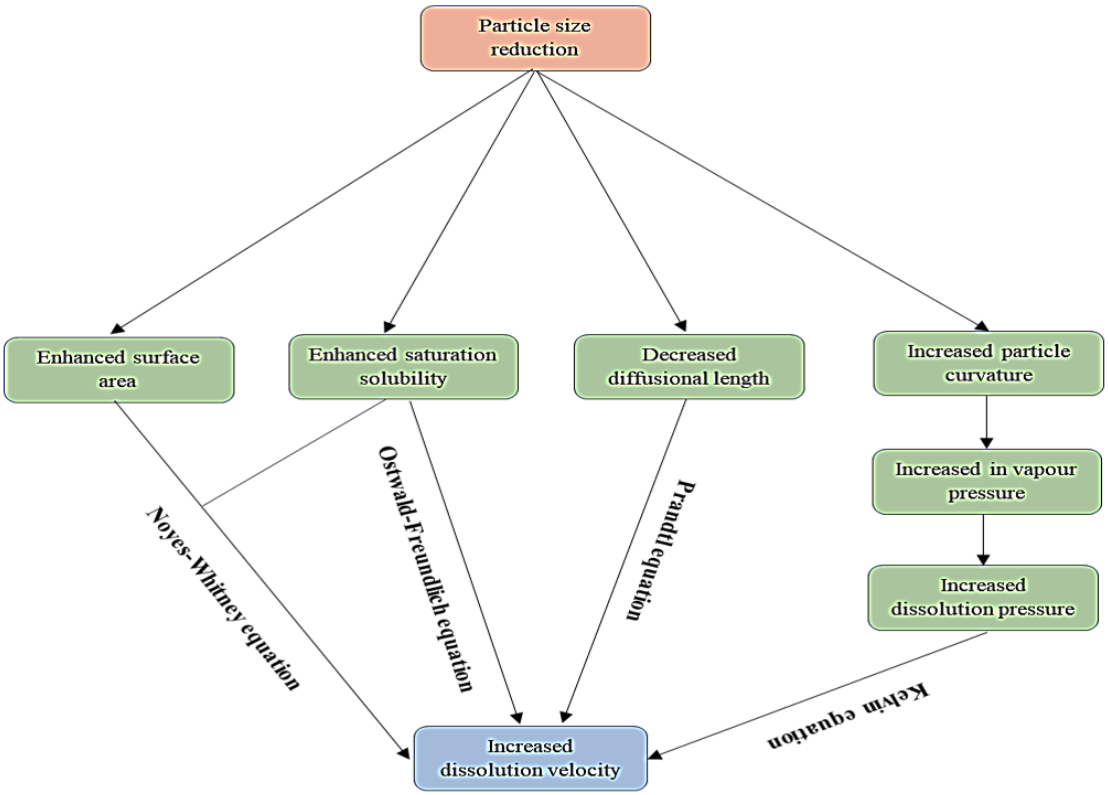

Figure 4: Inter-relationship between particle parameters and dissolution.

Laser diffraction works on the principle of measurement of angular variation in intensity of scattered light when a laser beam passes through a dispersed particulate sample. Large particles scatter light at small angles relative to the laser beam and small particles scatter light at large angles. The size range that can be measured varies with the instrument model and manufacturer. The Mastersizer 3000 model available from Malvern measures the size of the particles ranging from 0.01 µm to 3500 µm.

Dynamic Light Scattering measures the fluctuations in scattered light intensity caused by Brownian motion of particles. Analysis of these intensity fluctuations yields the velocity of the Brownian motion which is then related to particle size. The larger the particle or molecule, the slower the Brownian motion and vice versa for smaller particles. The Zetasizer Advance range of instruments available from Malvern can measure a particle size range of 0.3 nm-10 µm.

In Coulter Counter method, particles suspended in a weak electrolyte pass through a small diameter aperture positioned between 2 electrodes with an electric current. The passage of particle through the electrical sensing zone generates an electrical pulse. The height of the pulse is proportional to the particle size and sufficient to give a precise and absolute measurement of the particle. The typical size range that can be measured in Beckman Multisizer 4e Coulter Counter instrument is 0.2 um-1600 um.

The particle size of nanosuspensions meant for IV route of administration should be strictly controlled to ensure that the size is not more than 5 µm to avoid capillary blockade or emboli formation. The absolute value measurement techniques such as Coulter counter method is considered best suited to exercise strict controls during production of nanosuspensions.[66]

Particle Morphology

The two common instruments that are employed for determining the particle shape are Transmission Electron Microscope (TEM) and Scanning Electron Microscopy (SEM). While transmission electron microscope is a suitable technique for wet samples, the dried samples can be analysed by scanning electron microscopy. Analysis of dried sample using SEM techniques can reveal critical information related to changes in particle shape, size and signs of agglomeration as a result of solvent removal from the suspension during drying stage. Particle shape is crucial for Dry Powder Inhalers (DPIs), as different shapes result in different drag forces and terminal settling velocities, affecting lung deposition.[67]

Zeta Potential

The zeta potential is the potential difference between the dispersion medium and the stationary layer. The zeta potential indicates suspension stability, with an increase in it enhancing electrostatic stabilization. As the zeta potential approaches zero, van der Waals attraction overtakes electrostatic repulsion, leading to particle aggregation and poor physical stability. A minimum zeta potential of ±30 mV is required for stable suspensions though a potential of ±20 mV is considered sufficient for a

formulation that uses dual system consisting of electrostatic and steric stabilizer.[68]

Particle properties and dissolution velocity

The significance of nanosuspensions is that they enhance both saturation solubility and dissolution velocity. Particle properties and dissolution velocity are related in a number of ways, as illustrated by different theories and equations summarised below. As it is known, the decrease in particle size results in increased surface area and saturation solubility. This phenomenon is very well described by Ostwald-Freundlich equation.[69] Similarly, Noyes-Whitney equation is used to explain the enhancement in saturation solubility and dissolution velocity.[69] Also, the relationship describing decrease in particle size with resultant decrease in diffusional length is described using Prandtl equation.[69]

Besides size and surface area, particles of nano size range exhibit huge curvature compared to micro and coarse particles. This increase in curvature leads to increase in vapour pressure over curved surface, which in turn increases dissolution pressure in accordance with Kelvin equation.[69]

The relationship between particle properties and dissolution is diagrammatically represented in Figure 4.

pH Value

The pH is an important formulation parameter that can influence physicochemical stability characteristics of the formulation during processing and storage of the finished drug product. The important precaution that needs due consideration for the pH measurement of aqueous based formulation is the measurement at specified temperature once equilibrium attained, to avoid 'pH drift' that impacts measurement accuracy and repeatability.

Viscosity

The viscosity of nanosuspensions can be measured using a rotary viscometer such as Brookfield viscometer by varying temperature and shear rates. Viscosity is another important characteristic that can cause instability of nanosuspensions if not optimized during formulation development. Nanosuspension with optimum viscosity can minimize particle interactions and sedimentation velocity both of which can potentially facilitate particle agglomeration.

Sedimentation is a phenomenon in which particles of larger size settle naturally under the action of gravity. The velocity of settling particles (v) is explained by Stokes' law.[70]

Sedimentation is not problematic as long as rate of sedimentation is low and sediment formed can be easily redispersed. However, formation of hard cake or sediment which does not allow redispersion can have significant impact on quality, dose uniformity and biological performance of the drug molecule.

Stokes-Einstein equation[70] describes the impact of the temperature and viscosity on the physical stability of the nanosuspension formulation. As per Stokes-Einstein equation, an increase in viscosity helps in stabilizing nanosuspension by reducing the diffusion velocity whereas increase in temperature will cause decrease in viscosity and increase in diffusion coefficient favouring particle aggregation.

CONCLUSION

Employment of novel formulation technologies has become essential to tackle formulation challenges associated with new molecules that exhibit excellent therapeutic performance but have unfavourable biopharmaceutical properties, with poor aqueous solubility as the major concern. The use of drug nanosuspension is one of the most promising approaches to increase the drug solubility and bioavailability, which are critical for realising the optimum therapeutic benefits of drug formulation. Among various techniques employed for preparing nanosuspensions, top-down methods occupy the driving seat not only because of significant technological advancements but also because of the industrial applicability, scalability and reproducibility of the processes for commercial scale production of nanosuspensions. Also, the rapid introduction of advanced characterization techniques coupled with the depth in understanding the stabilization methods and biological mechanisms is helping formulation scientists design and develop more effective and target oriented nanosuspensions with scope expansion to include large molecules such as proteins as well.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BCS: Biopharmaceutical Classification System; **IDD-P:** Insoluble Drug Delivery-Particles; **USA:** United States of America; **QbD:** Quality by Design; **USFDA:** United States Food and Drug Administration; **ICH:** International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; **IV:** Intravenous.

SUMMARY

Nanosuspensions have unique properties due to their size, which can enhance solubility, adapt to surface modification and mucoadhesion for drug targeting. However, key aspects to consider when designing nanosuspensions include stability in liquid and solid states and dispersibility without aggregation. Among different techniques available to design and develop nanosuspension formulations, top-down methods such as media milling and high-pressure homogenization have drawn interest from researchers in both academia and the pharmaceutical industry due to their high level of adoptability and scalability. The

application of top-down methods has resulted in the development and commercialization of multiple innovative drug products using oral and parenteral routes of administration globally.

REFERENCES

1. Williams HD, Trevaskis NL, Charman SA, Shanker RM, Charman WN, Pouton CW, *et al.* Strategies to address low drug solubility in discovery and development. Pharmacol Rev. 2013;65(1):315-499.
2. Chogale MM, Date AA, Ghodake VN, Payghan SA, Disouza JI, Patravale VB. Nanosuspensions: Formulation, Characterization and Applications. In: Raj K. Keservani, Anil K. Sharma, editors. Nanodisperisons for Drug Delivery. New Jersey and Ontario: Apple Academic Press Inc; 2019. p. 109-10.
3. Dressman JB, Amidon GL, Reppas C, Shah VP. Dissolution testing as a prognostic tool for oral drug. Pharm Res. 1998;15(1):11-22.
4. Sathe P, Lionberger RA, Lee SL, Yu LX, Zhang D (Doris). Regulatory aspects of Dissolution for Low Solubility Drug Products. In: Ron Liu, editor. Water-Insoluble drug Formulation. Florida: CRC Press; 2018. p. 113-24.
5. Yadollahi R, Vasilev K, Simovic S. Nanosuspension technologies for delivery of poorly soluble drugs. J Nanomater. 2015;1-13.
6. Khadka P, Ro J, Kim H, Kim I, Kim JT, Kim H, *et al.* Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. Asian J Pharm Sci. 2014;9(6):304-16.
7. Jacob S, Nair AB, Shah J. Emerging role of nanosuspensions in drug delivery systems. Biomater Res. 2020;24(3):1-16.
8. Kanikkannan N. Technologies to Improve the Solubility, Dissolution and Bioavailability of Poorly Soluble Drugs. J Anal Pharm Res. 2018;7(1):1-6.
9. Qiao Y, Wei Z, Qin T, Song R, Yu Z, Yuan Q, *et al.* Combined nanosuspensions from 2 natural active ingredients for cancer therapy with reduced side effects. Chin Chem Lett. 2021;32(9):2877-81.
10. Riaz M. Review article: stability and uses of liposomes. Pak J Pharm Sci. 1995;8(2):69-79.
11. Jadhav KR, Shaikh IM, Ambade KW, Kadam VJ. Applications of microemulsion based drug delivery system. Curr Drug Deliv. 2006;3(3):267-73.
12. Leuner C, Dressman J. Improving drug solubility for oral delivery using solid dispersions. Eur J Pharm Biopharm. 2000;50(1):47-60.
13. Challa R, Ahuja A, Ali J, Khar RK. Cyclodextrins in drug delivery: an updated review. AAPS PharmSciTech. 2005;6(2):E329-57.
14. Kostarelos K. The emergence of nanomedicine: a field in the making. Nanomedicine (Lond). 2006;1(1):1-3.
15. Keck CM, Müller RH. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. Eur J Pharm Biopharm. 2006;62(1):3-16.
16. Pınar SG, Oktay AN, Karakucuk AE, Celebi N. Formulation Strategies of Nanosuspensions for Various Administration Routes. Pharmaceutics. 2023;15(5):1-28.
17. Vinchhi P, Patel JK, Patel MM. High-Pressure Homogenization Techniques for Nanoparticles. In: Jayvadan K. Patel, Yashwant V. Pathak, editors. Emerging Technologies for Nanoparticle Manufacturing. Switzerland: Springer; 2021. p. 263-9.
18. Moschwitzer J, Müller RH. New method for the effective production of ultrafine drug nanocrystals. J Nanosci and Nanotechnol. 2006;6(9-10):3145-53.
19. Junghanns JU, Müller RH. Nanocrystal technology, drug delivery and clinical applications. Int J Nanomedicine. 2008;3(3):295-309.
20. He J, Han Y, Xu G, Yin L, Ngandeu Neubi M, Zhou J, *et al.* Preparation and evaluation of celecoxib nanosuspensions for bioavailability enhancement. RSC Adv. 2017;7(22):13053-64.
21. Xie J, Luo Y, Liu Y, Ma Y, Yue P, Yang M. Novel redispersible nanosuspensions stabilized by co-processed nanocrystalline cellulose-sodium carboxymethyl starch for enhancing dissolution and oral bioavailability of baicalin. Int J Nanomedicine. 2019;14:353-69.
22. Dibaei M, Rouini MR, Sheikholeslami B, Gholami M, Dinarvand R. The effect of surface treatment on the brain delivery of curcumin nanosuspension: *In vitro* and *in vivo* studies. Int J Nanomedicine. 2019;14:5477-90.
23. Hedaya M, Bandarkar F, Nada A. *In vitro* and *in vivo* Evaluation of Ibuprofen Nanosuspensions for Enhanced Oral Bioavailability. Med Princ Pract. 2021;30(4):361-8.
24. Moschwitzer J, Muller R. Drug Nanocrystals-The Universal Formulation Approach for Poorly Soluble Drug. In: Thassu D, Deleers M, Pathak Y, editors. Nanoparticulate Drug Delivery Systems. New York: Informa Healthcare; 2007. p. 71-88.
25. Radke M. Pure Drug Nanoparticles for the Formulation of Poorly Soluble Drugs. New Drugs 2001;3:62-8.
26. Kavinila S, Nimbkar S, Moses JA, Anandharamakrishnan C. Emerging applications of microfluidization in the food industry. J Agric Food Res. 2023;12:1-10.
27. O., Ocampo-Salinas I., I., Tellez-Medina D., C., Jimenez-Martinez, G., Davila-Ortiz. Application of High Pressure Homogenization to Improve Stability and Decrease Droplet Size in Emulsion-Flavor Systems. International Journal of Environment, Agriculture and Biotechnology. 2016;1(4):646-62.
28. Jia Y, Jiang Y, He Y, Zhang W, Zou J, Magar KT, *et al.* Approved Nanomedicine against Diseases. Pharmaceutics. 2023;15(3):1-32.
29. Gulbag Pinar S, Celebi N. Optimization and evaluation of cyclosporine a nanosuspension stabilized by combination stabilizers using high pressure homogenization method. J Res Pharm. 2019;23(6):1009-21.
30. Karakucuk A, Celebi N, Teksin ZS. Preparation of ritonavir nanosuspensions by microfluidization using polymeric stabilizers: I. A Design of Experiment approach. Eur J Pharm Sci. 2016;95:111-21.
31. Verma S, Lan Y, Gokhale R, Burgess DJ. Quality by design approach to understand the process of nanosuspension preparation. Int J Pharm. 2009;377(1-2):185-98.
32. Li L, Liu Y, Wang J, Chen L, Zhang W, Yan X. Preparation, *in vitro* and *in vivo* evaluation of bexarotene nanocrystals with surface modification by folate-chitosan conjugates. Drug Deliv. 2016;23(1):79-87.
33. Sharma S, Verma A, Teja BV, Shukla P, Mishra PR. Development of stabilized Paclitaxel nanocrystals: *In vitro* and *in vivo* efficacy studies. Eur J Pharm Sci. 2015;69:51-60.
34. Zhang Y, Zhang J. Preparation of budesonide nanosuspensions for pulmonary delivery: Characterization *in vitro* release and *in vivo* lung distribution studies. Artif Cells Nanomed Biotechnol. 2016;44(1):285-9.
35. Loh ZH, Samanta AK, Sia Heng PW. Overview of milling techniques for improving the solubility of poorly water-soluble drugs. Asian J Pharm Sci. 2015;10(4):255-74.
36. Merisko-Liversidge E, Liversidge GG, Cooper ER. Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur J Pharm Sci. 2003;18(2):113-20.
37. Patel Vivek P, Patel Dhara V, Patel Jayvadan K. Nanocrystallization and Nanoprecipitation Technologies. In: Jayvadan K. Patel, Yashwant V. Pathak, editors. Emerging Technologies for Nanoparticle Manufacturing. Cham, Switzerland: Springer; 2021. p. 45-50.
38. Kenth S, Sylvestre JP, Fuhrmann K, Meunier M, Leroux JC. Fabrication of paclitaxel nanocrystals by femtosecond laser ablation and fragmentation. J Pharm Sci. 2011;100(3):1022-30.
39. Besner S, Kabashin A V., Meunier M. Fragmentation of colloidal nanoparticles by femtosecond laser-induced supercontinuum generation. Appl Phys Lett. 2006;89(23):1-3.
40. Kenth S, Sylvestre JP, Fuhrmann K, Meunier M, Leroux JC. Fabrication of Paclitaxel Nanocrystals by Femtosecond Laser Ablation and Fragmentation. J Pharm Sci. 2011;100(3):1022-30.
41. Sylvestre JP, Tang MC, Furtos A, Leclair G, Meunier M, Leroux JC. Nanonization of megestrol acetate by laser fragmentation in aqueous milieu. J Control Release. 2011;149(3):273-80.
42. Ding W, Sylvestre JP, Leclair G, Meunier M. Laser Fragmentation as an Efficient Size-Reduction Method for Pulmonary Drug Discovery: Proof-of-Concept Study of Beclomethasone Dipropionate. IJTAN. 2012;1(1):99-104.
43. Ding W, Sylvestre JP, Bouvier E, Leclair G, Meunier M. Ultrafast laser processing of drug particles in water for pharmaceutical discovery. Appl Phys A Mater Sci Process. 2014;114(1):267-76.
44. Gera T, Nagy E, Smausz T, Budai J, Ajtai T, Kun-Szabó F, *et al.* Application of pulsed laser ablation (PLA) for the size reduction of non-steroidal anti-inflammatory drugs (NSAIDs). Sci Rep. 2020;10(1):1-13.
45. Singh A, Kutscher HL, Bulmahn JC, Mahajan SD, He GS, Prasad PN. Laser ablation for pharmaceutical nanoformulations: Multi-drug nanoencapsulation and theranostics for HIV. Nanomedicine. 2020;25:1-22.
46. Kipp James E, Wong Joseph, Doty Mark J, Werling Jane, Rebbeck Christine L, Brynjelsen Sean, inventors; Baxter International Inc., assignee. Method for preparing submicron particle suspensions. United States patent US6884436B2. 2005 Apr 26.
47. Kipp James E, Wong Joseph, Doty Mark J, Rebbeck Christine L, inventors; Baxter International Inc., assignee. Microprecipitation method for preparing submicron suspensions. United States patent US7037528B2. 2006 May 2.
48. Keck C, Kobierski S, Mauludin R, Muller RH. Second generation of drug nanocrystals for delivery of poorly soluble drugs: smartCrystals technology. Dosis. 2008;24(2):124-8.
49. Salazar J, Muller RH, Möschwitzer JP. Combinative Particle Size Reduction Technologies for the Production of Drug Nanocrystals. J Pharm (Cairo). 2014;1-14.
50. Petersen Rolf, inventor; AbbVie Deutschland GmbH and Co KG, assignee. Nanocrystals for use in topical cosmetic formulations and method of production thereof. United States patent US9114077B2, 2015.
51. Rabinow B, Kipp J, Papadopoulos P, Wong J, Glosson J, Gass J *et al.* Itraconazole IV nanosuspension enhances efficacy through altered pharmacokinetics in the rat. Int J Pharm. 2007;339(1-2):251-60.
52. Shelar DB, Pawar SK, Vavia PR. Fabrication of isradipine nanosuspension by anti-solvent microprecipitation-high-pressure homogenization method for enhancing dissolution rate and oral bioavailability. Drug Deliv Transl Res. 2013;3(5):384-91.
53. Salazar J, Müller RH, Moschwitzer JP. Performance comparison of 2 novel combinative particle-size-reduction technologies. J Pharm Sci. 2013;102(5):1636-49.
54. Attari, Z., Kalvakuntla, S., Reddy, M. S., Deshpande, M., Rao, C. M., and Koteshwara, K. B. Formulation and characterisation of nanosuspensions of BCS class II and IV drugs by combinative method. J.Exp.Nanosci. 2016;11(4), 276-288.
55. Morakul B, Suksiriworapong J, Leanpolchareanchai J, Junyaprasert VB. Precipitation-lyophilization-homogenization (PLH) for preparation of clarithromycin nanocrystals: Influencing factors on physicochemical properties and stability. Int J Pharm. 2013;457(1):187-96.

56. Shi-Ying J, Jin H, Shi-Xiao J, Qing-Yuan L, Jin-Xia B, Chen HG, *et al.* Characterization and evaluation *in vivo* of baicalin-nanocrystals prepared by an ultrasonic-homogenization-fluid bed drying method. Chin J Nat Med. 2014;12(1):71-80.
57. Yu X. Preparation and Formulation of Novel Drug nanoparticles Based on High efficient solubilization [master's thesis]. Qingdao [China]: Qingdao University of Science and Technology; 2021.
58. Zuo X. Preparation of New Nanopharmaceutical Combination Method and Its Preparation [master's thesis]. Qingdao [China]: Qingdao University of Science and Technology; 2019.
59. Zhang H, Meng Y, Wang X, Dai W, Wang X, Zhang Q. Pharmaceutical and pharmacokinetic characteristics of different types of fenofibrate nanocrystals prepared by different bottom-up approaches. Drug Deliv. 2014;21(8):588-94.
60. Liu D, Xu H, Tian B, Yuan K, Pan H, Ma S, *et al.* Fabrication of carvedilol nanosuspensions through the anti-solvent precipitation-ultrasonication method for the improvement of dissolution rate and oral bioavailability. AAPS PharmSciTech. 2012;13(1):295-304.
61. Lu Y, Wang ZH, Li T, McNally H, Park K, Sturek M. Development and evaluation of transferrin-stabilized paclitaxel nanocrystal formulation. J Control Release. 2014;176(1):76-85.
62. Latif R, Makar RR, Hosni EA, El Gazayerly ON. The potential of intranasal delivery of nanocrystals in powder form on the improvement of zaleplon performance: *in vitro, in vivo* assessment. Drug Dev Ind Pharm. 2021;47(2):268-79.
63. Zhu Y, Fu Y, Zhang A, Wang X, Zhao Z, Zhang Y, *et al.* Rod-shaped nintedanib nanocrystals improved oral bioavailability through multiple intestinal absorption pathways. Eur J Pharm Sci. 2022;168:1-12.
64. Shete G, Jain H, Punj D, Prajapat H, Akotiya P, Kumar Bansal A. Stabilizers used in nano-crystal based drug delivery systems. J Excip Food Chem. 2014;5(4):184-209.
65. Wang Y, Zheng Y, Zhang L, Wang Q, Zhang D. Stability of nanosuspensions in drug delivery. J of Control Release. 2013;172(3):1126-41.
66. Patravale VB, Date AA, Kulkarni RM. Nanosuspensions: a promising drug delivery strategy. J Pharm Pharmacol. 2004;56(7):827-40.
67. Chogale MM, Ghodake VN, Patravale VB. Performance parameters and characterizations of nanocrystals: A brief review. Pharmaceutics. 2016;8(3):1-18.
68. Muller RH, Jacobs C, Kayser O. Nanosuspensions as particulate drug formulations in therapy. Rationale for development and what we can expect for the future. Adv Drug Deliv Rev. 2001;47(1):3-19.
69. Junyaprasert VB, Morakul B. Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs. Asian J Pharm Sci. 2015;10(1):13-23.
70. Li J, Wang Z, Zhang H, Gao J, Zheng A. Progress in the development of stabilization strategies for nanocrystal preparations. Drug Deliv. 2021;28(1):19-36.

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