Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i4.3171

NANOCRYSTALS: A TOOL FOR ENHANCING DRUG DISSOLUTION

Sadhu Venkateswara Rao[1], Gopu Navyatha[1], Parakat Tanmai Meenakshi[1], Akunuri Sai Divya[1], Chandolu Lakshmi Akhila Jahnavi[1], Kantamneni Padmalatha[2]

[1*]Department of Pharmaceutics, Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada, Andhra Pradesh-521102, India. email- venkateshsadhu@gmail.com
[2]Department of Pharmacology, Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada, Andhra Pradesh-521102, India.

***Corresponding Author:** Dr. Sadhu Venkateswara Rao
*Professor & HoD, Department of Pharmaceutics, Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada, Andhra Pradesh, India.

Abstract
Poor aqueous solubility is a significant challenge in the formulation and delivery of many pharmaceutical compounds, leading to suboptimal bioavailability and therapeutic efficacy. Nanocrystals have emerged as a promising approach to address this issue by enhancing drug dissolution and improving drug delivery systems. This review provides an in-depth analysis of the role of nanocrystals in enhancing drug dissolution and their potential as a tool for improving drug formulations and explaining the principles of nanocrystal technology, including the media milling, preparation method and homogenization method, and the use of stabilizers and surfactants to prevent aggregation. The applications of nanocrystals in different drug delivery systems are explored, including oral, parenteral, and topical routes. In conclusion, nanocrystals represent a powerful tool for enhancing drug dissolution and improving drug delivery systems for poorly water-soluble drugs. Their ability to enhance bioavailability and therapeutic efficacy holds great promise for advancing pharmaceutical development.

Key Words: Nanocrystals, Dissolution, Stabilizers and Bioavailability.

INTRODUCTION
It is believed that 40% or more of active compounds found by combinatorial screening techniques have poor water solubility. Poor solubility is a barrier for screening novel compounds for pharmacological activity at the very beginning, as well as throughout formulation development and clinical testing. This indicates that smart technological formulation techniques are definitely needed to enhance the bioavailability of these inadequately soluble drugs. The term "making such drugs bioavailable" refers to their ability to be administered intravenously or orally with a suitable level of absorption. (1) A number of techniques have been utilized for quite some time to improve drug solubility. These include solubilization by surfactants, complex formation (e.g., cyclodextrin, macromolecules), self-emulsifying drug delivery systems (SEDDS), microemulsions, and, most importantly for oral administration, micronization of drug powders. (2) The term "micronization" refers to the process of reducing drug powders to an average particle size of 1–10m. However, many modern pharmaceuticals are notoriously weakly soluble,

rendering micronization inadequate. An increase in surface area and the resulting increase in dissolving speed is not enough to alleviate the bioavailability problems associated with very poorly soluble medicines of biopharmaceutical specification class II. The consequent next step after micronization was nanonization. At the start of the 21st century, drug nanocrystals were developed as a more efficient strategy for increasing drug solubility and the rate of dissolution. Nanocrystals with typical diameters between 200 nm and 600 nm are produced by nanonizing the drug powder rather than micronizing it. Chemically unstable drugs can be stabilized by encapsulating them in drug nanocrystals. When paclitaxel is formulated as a nanosuspension, it can be protected against degradation.(3) When the chemically unstable medication omeprazole was made into a nanosuspension, a similar result was seen; the stability was significantly better than the aqueous solution.(4) Drug nanocrystals are pure solid particles of a drug with a diameter of less than 1000 nm. The components of a nanosuspension are the drug nanocrystals, a liquid dispersion medium, and the stabilizing components (which may include surfactants and/or polymeric stabilizers). Aqueous solutions, water, and non-aqueous media are all acceptable dispersion media. The term "drug nanocrystals" implies that the discrete particles are in a crystalline state, however depending on the manufacturing process, they could also be partially or completely amorphous. Drug nanocrystals must be separated from polymeric nanoparticles, which consists of an incorporated drug and a polymeric matrix. Drug nanocrystals are devoid of any matrix components.

INCREASING DISSOLUTION — THEORETICAL ASPECTS

Increased saturation solubility and faster dissolving rate are the distinguishing features of drug nanocrystals. In most cases, the saturation solubility (cs) of a medication is presented as a constant that is dependent on both the solvent and the temperature. Particles of a drug must be at least a few micrometers in size to meet this condition. The solubility of a drug can be enhanced by reducing the particle size to the nanoscale range. According to the Nernst-Brunner/Noyes-Whitney equation, the surface area accessible for dissolution is directly proportional to the dissolving rate of a solid API. (5)

$$\frac{dx}{dt} = A.\frac{D}{h}\left(Cs - \frac{xd}{v}\right)$$

for which Xd=amount dissolved, dX/dt=dissolution rate, Cs=saturation solubility, V=volume of dissolvable fluid, A=particle surface area, D=diffusion coefficient, h=effective boundary layer thickness. Pharmaceutical active ingredient (API) micronization has been widely used on the basis of this idea to increase the oral bioavailability of drugs. Dissolving rate can be improved by increasing the effective particle surface area, which is achieved by decreasing particle size to the sub-micron region.(6) In the case of aprepitant, for example, the nanocrystal dispersion with 120-nm particles has a surface area that is 41.5 times larger than the normal 5μm suspension.(7) Additionally, as predicted by the Prandtl equation, the thickness of the diffusion layer (h) will also be reduced, leading to an even faster rate of dissolution.(8) In addition to the improved dissolving rate, the nanosized API is also predicted to have an increased saturation solubility (9), as described by the Freundlich– Ostwald equation:

$$S = S\exp\left(\frac{2\gamma\mu}{\gamma\rho T}\right)$$

where S= Nanosized API saturation solubility, S8=saturation solubility of an infinitely large API crystal, is the crystal-medium interfacial tension, M is the molecular weight of a compound, r is the radius of particle, is the density, R is a gas constant and T is the temperature. The above equation predicts a 10-15% improvement in solubility at a particle size of 100 nm for a substance with a molecular weight of 500=1 g/mL and an interfacial tension between the crystal and the intestinal fluid of 15-20 mN m-1. However, in practice, the solubility increase is typically much larger; for instance, Muller and Peters found that decreasing the particle size of an insoluble antimicrobial medication from 2.4 m to 800 or 300 nm increases the drug's solubility by 50%(9)

Because of this enhanced solubility and dissolution rate, nanosuspensions often achieve significantly higher exposure levels than micronized API suspensions, even when using the same surfactants. Finally, it is expected that the dissolving rates of nanosuspension formulations will be higher than those of micronized suspensions due to the increased surface wetness generated by the surfactants.

METHODS FOR PRODUCTION OF NANOCRYSTALS

High-pressure homogenization,milling and precipitation are the most common approaches for producing drug nanocrystals. It is widely accepted that the production of drug nanocrystals is important for increasing the bioavailability of poorly soluble pharmaceuticals. Extensive investigation into cutting-edge technologies has yielded an abundance of alternate routes for producing drug nanocrystals. Companies like Dow Chemical, who are not traditionally associated with the pharmaceutical industry, have entered the market for poorly soluble medications by developing technology to improve their solubility. The following procedures using supercritical fluids are included just as an exhaustive list. These include evaporative precipitation into an aqueous solution (EPAS), spray freezing into liquid (SFL)and aerosol solvent extraction (ASES), as well as rapid expansion from supercritical to aqueous solution (RESAS), rapid expansion of supercritical solution (RESS) and solution-enhanced dispersion by supercritical fluids (SEDS). (Müller and Bleich 1996; Lee et al 2005).

Media Milling Process

Milling media, dispersion medium (often water), stabilizer, and drugs are loaded into a milling chamber to create nanocrystalline dispersions utilizing NanoCrystals® technology. Shear pressures and impaction forces created by the motion of the milling media break the drug particles down to smaller sizes. Milling media can be either small milling pearls or larger milling balls. In a media mill, the grinding and dispersion action (i.e., leading to smaller particles) is greatly enhanced by decreasing the size of the grinding media, which in turn exponentially increases the number of contact sites. Beads of strongly cross-linked polystyrene resin cover ceramic (cerium- or yttrium-stabilized zirconium dioxide) beads, stainless steel, glass, or glass pearls. Erosion caused by the milling material itself is an issue with pearl milling technology. [(10)] When milling with glass, buchmann and co. reported the creation of glass microparticles. Highly cross-linked polystyrene resin was employed to cover the milling beads, reducing the number of contaminants introduced through media erosion. [(11)] Product adhering to the inside of the milling system is an ongoing issue. Together, the chamber and the milling beads make up the inner surface area. This product adhesion results in a loss of product even in recirculation systems. When only a little amount of a new chemical entity (NCE) is processed, this unwanted medication loss can become a problem. This method is used for manufacturing a wide range of commercially available formulations, including-Wyeth introduced Rapamune, the first medicine to contain sirolimus NanoCrystals, in the year 2000. When compared to the Rapamune® solution, the bioavailability of the coated tablets is 27% higher while also being more convenient for administration. [(12)] This is an example of the comparison between two methods of formulation. The oral solution illustrates the principles of cosolvents and surfactants, while the tablets give a decent performance of a particle size reduction technique. Emend® is the second product to implement Nanocrystal technology. It hit markets after being introduced by Merck in 2003. Sodium dodecylsulfate, microcrystalline cellulose, sucrose, and nanocrystalline aprepitant are the pellets inside of the Emend® capsule.[(13)] Abbott's nanocrystalline fenofibrate tablet, Tricor, was released in 2004. In late 2005, a fourth medication, Megaace ES, an oral suspension containing megestrol acetate, was released for the treatment of anorexia and cachexia caused by HIV.

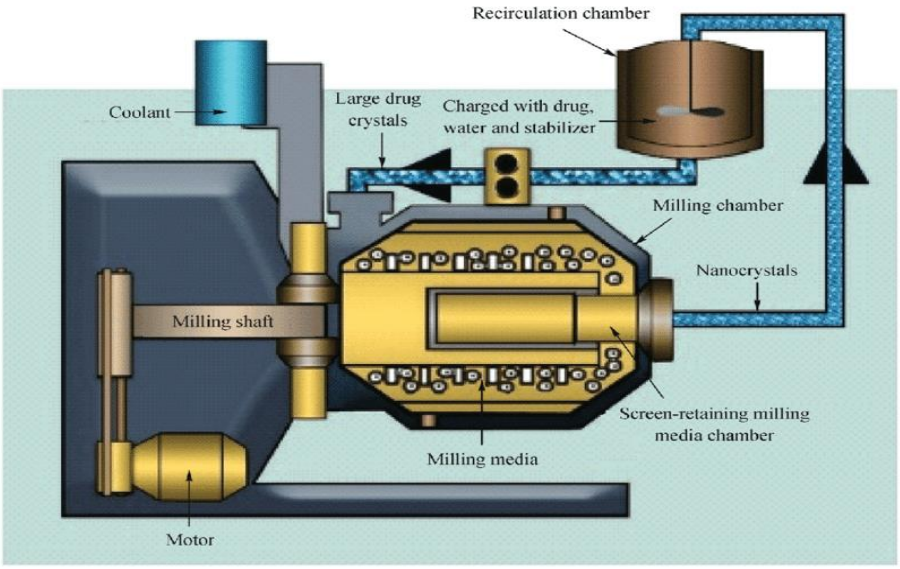

Fig (1). Media milling process

Precipitation Method

Sandoz (now Novartis), the owner of the IP, developed this technique, which is also known as hydrosol technology. A drug that isn't very water-soluble can be dissolved in an organic medium that is miscible with water. Transferring this solution into a non-solvent, like water, causes the nanocrystals of the drug to precipitate out. The created nanoparticles need to be stabilized to prevent micrometer-scale crystal formation, which is a disadvantage of this method. Newly synthesized or discovered drugs that are poorly soluble in water and organic media provide a problem because they must be soluble in at least one solvent. Particle size is best maintained with lyophilization. [(14)] Polymeric growth inhibitors, ideally those soluble in aqueous solution, are another option for maintaining the size of precipitated nanocrystals. Particle growth can be slowed by increasing the viscosity of the aqueous phase. There is no product available in the market that uses this technology at present time.

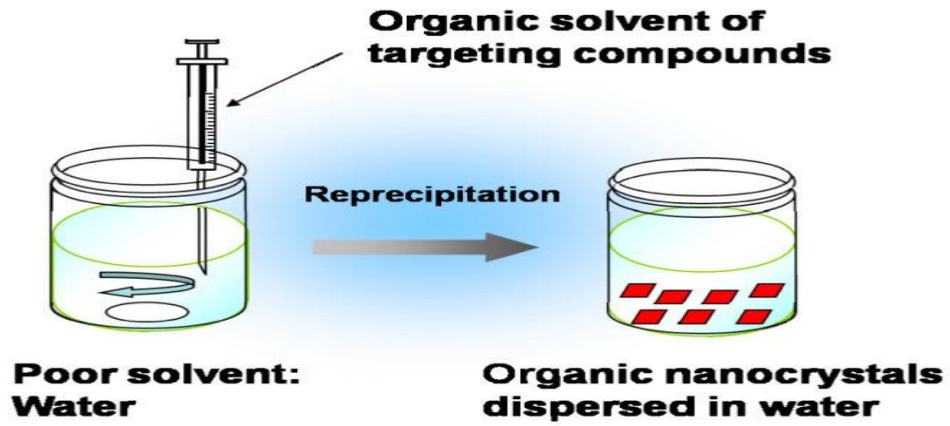

Fig (2). Precipitation method

Homogenization Methods

Microfluidizer Technology (IDD-P™ technology)

The microfluidizer is a jet stream homogenizer, which means that it uses high pressures (up to 4000 bar) to cause a frontal collision between two fluid streams traveling at high speeds (up to 1000m/sec). Due to the tremendous shear pressures, particle collisions, and turbulent flow, particles are broken down to the nanoscale range. [(15)] Cavitation, caused by the high pressure

applied and the fast streaming velocity of lipids, also aids in size reduction. Particle size must be stabilized using phospholipids or other surfactants and stabilizers. One major drawback of this approach is the length of time needed to produce results. To reduce particle size sufficiently, it may take as many as 50 to 100 time-consuming passes.[16] Skye Pharma Canada, Inc. (previously RTP, Inc.) applies this principle for its IDD-P™ technology to produce submicron particles of poorly soluble drugs [17]

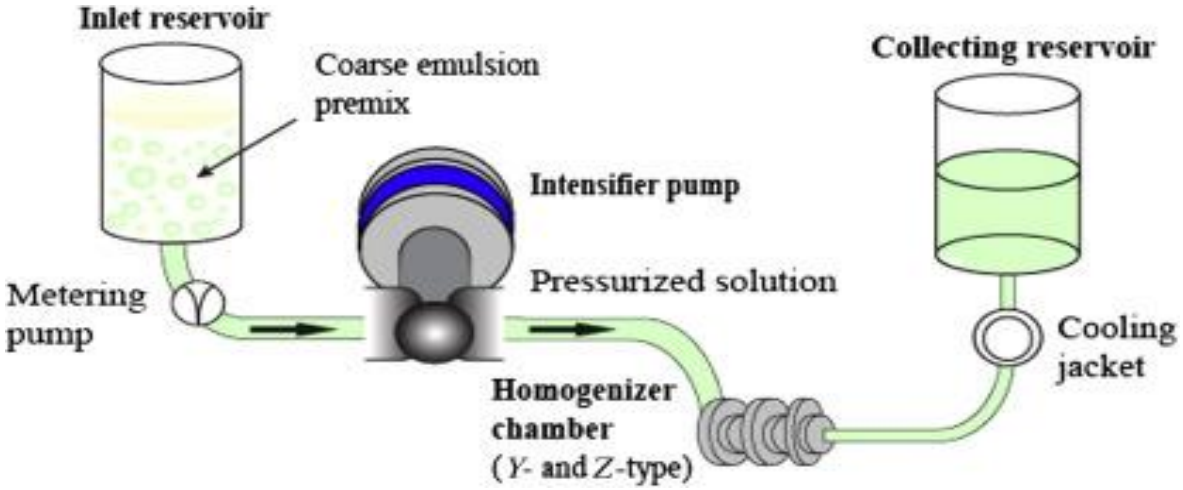

Fig (3). Microfluidizer technology (IDD-P™ technology*)*

Piston – Gap Homogenization in Water (DISSOCUBES®)

Drug nanocrystals can also be made using high-pressure homogenization with piston gap homogenizers. When it comes to homogenization temperature and dispersion media, Dissocubes® and Nanopure® diverge. Water was used as the medium for dispersing the suspensions. Up to 2000 bar of pressure can be generated by a piston in a big bore cylinder. A relatively small ring gap is used to exert force on the suspension. The gap width is typically 3-15 micrometers at pressures of 1500-150 bar. The Bernoulli equation predicts a fast stream rate in the crack. [18] The static pressure on the liquid is reduced when the diameter of the cylinder is reduced from a large bore cylinder (e.g., 3 cm) to the homogenization gap, increasing the dynamic pressure (streaming velocity). As the suspension exits the void and returns to atmospheric pressure (cavitation), the liquid boils and gas bubbles form, only to burst as the pressure changes. Smaller particles are created as shock waves travel through the gas during bubble creation and collapse. According to the patent [19], the size reduction was accomplished using a process called cavitation. For the purpose of creating nanosuspensions, manufacturers including APV Gaulin, Avestin, and Niro Soavi offer piston-gap homogenizers. Beginning at the tail end of the 1990s, Skye Pharma PLC made use of the newly acquired technology in the form of improved formulations. There are some drawbacks when employing water as a dispersion medium. Drugs that are susceptible to water may hydrolyze, and drying procedures may encounter difficulties. Drugs with low melting points or those that are thermolabile require relatively expensive techniques, like lyophilization, to completely remove the water. For these reasons, the Dissocubes® technology excels when the resulting nanosuspension needs no additional processing, including drying steps, before being put to use. DissoCubes are a multi-drug product of high-pressure homogenization. It is possible to develop a nanosuspension of any of the drugs that have been studied so far. Some drugs that fall into this category include RMKP22, carbamazepine (unpublished data), buparvaquone, aphidicolin, cyclosporine, paclitaxel, RMBB98, azodicarbonamide, prednisolone, and others.[19]

Nanopure® Technology

Nanopure technology (Pharma Sol GmbH, Germany) uses a high-pressure homogenization process to transform pharmaceuticals that aren't water-soluble into drug nanocrystals. A high-

pressure homogenizer is used to turn the coarse drug powder into drug nanoparticles with mean diameters of 200 to 600 nm by dispersing it in a surfactant solution. The drug powder is first pre-suspended in a nonaqueous medium (like PEG 600 or Miglyol 812) or a water-reduced mixture (like water and ethanol), and then homogenized in a piston-gap homogenizer. The Micron Lab 40 (APV Deutschland GmbH) is a great example of a device designed for use in a scientific setting. Direct capsule filling is possible with the use of suspensions made with nonaqueous dispersion media, such as PEG or oils, eliminating the need for the intermediate step necessitated by the use of pure aqueous nanosuspension.. Homogenization can be carried out using nanopure technology in nonaqueous phases or phases with little water content. And even below the freezing point of water, homogenization was comparable to or more effective than cavitation, which was more prominent at higher temperatures.[20]

Combination Technologies

Nanoedge® Technology

Nanoedge technology (Baxter Healthcare Corporation, Deerfield, IL) has reported on a formulation strategy for pharmaceuticals that are poorly water-soluble. It's a helpful method for substances with high melting points, logPs, and octanol-water partition coefficients. Microprecipitation, lipid emulsions, and direct homogenization are the backbone of this technique. In microprecipitation, the medication is initially dissolved in a solvent that is miscible with water. Microprecipitation requires the medicine to be dissolved in a solvent that is miscible with water. The solution is then presuspended by combining it with a second solvent. After this pre-suspension is energized, particles with an effective particle size of 400 nm to 2 are formed. Impact, shear, or cavitation forces, such as those generated by sonication, homogenization, countercurrent flow homogenization, or microfluidization, are introduced as part of the energy-adding process. If water-for-injection is included in the formulation and an appropriate procedure for solution sterilization is employed, a drug suspension made using these approaches can be given intravenously. Small particle sizes [1000nm (volume weighted mean)], high drug loading [10-200mg/ml], no co solvent, lower surfactant levels, and the use of safe, well-tolerated surfactants are all made possible by nanoedge technology. [21] NANOEDGE® technique is particularly well suited for pharmaceuticals with low toxicity that are soluble in non-aqueous environments, such as N-methyl-2-pyrrolidinone. [22]

Nanopure® Xp Technology

The ability to use "normal" manufacturing settings for production on a massive scale is a significant benefit of this technology. Particles with a diameter of less than 100 nm can be created using the pre-treatment and homogenization steps of Pharma Sol's Nanopure XP technology. "(Müller and Moeschwitzer 2005)". Drug nanocrystals that are 50 nm in size and less are noticeably smaller than the wavelength of visible light, which causes the nanosuspensions to be translucent.

Other Techniques

Rapid – expansion from a liquefied – gas solution

The RESS method takes advantage of the extreme solvating capability of supercritical fluids. When the supercritical fluid carrying the solute is expanded in a supersonic free jet, it rapidly changes from a supercritical to a gaseous state. Particles are created as a result of the high supersaturation caused by the phase transition. Since the solvent becomes a dilute gas during expansion, the final product of the RESS method is solvent-free. The enhanced bioavailability of griseofulvin as a result of RESS was confirmed by dissolution studies utilizing the Stricker model. RESS-made griseofulvin has a dissolving rate almost twice as fast as that of ordinary micronized material. [23] According to modeling results, it should be able produce particles with a diameter smaller than 50 nm. Young et al. produced cyclosporine nanoparticles with this method that ranged in size from 500 to 700 nm. Tween80 solution was used as a surfactant to stop the

flocculation and agglomeration of nanoparticles. The cyclosporine particles created by this method have been shown to be stable at drug doses of up to 6.2 and 37.5 mg/ml in 1.0 and 5% (w/w), Tween80 solutions, respectively. [24] The RESS approach combined with high-pressure homogenization was utilized by Pace et al. to create a physically stable nanosuspension. This technique involves dissolving the poorly soluble medications and surface modification in a compressed, liquefied gas solvent before expanding it into an aqueous solution including surfactant. In order to produce a stable nanosuspension, the suspension that had just been created was next put through a high-pressure homogenization procedure. [25]

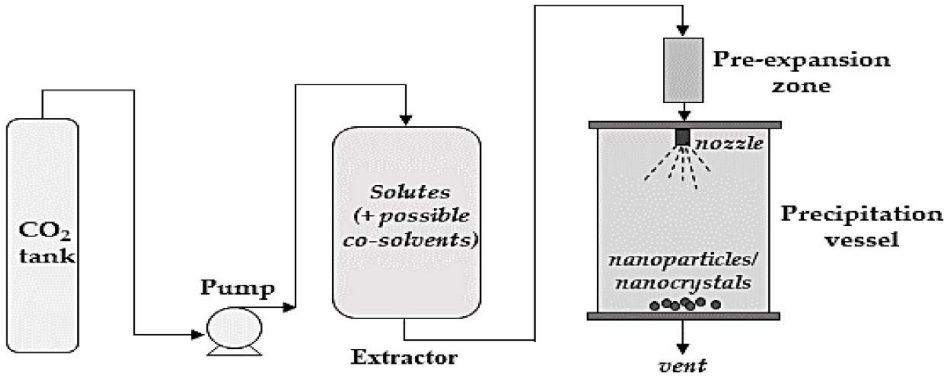

Fig (4). Rapid – expansion from a liquefied – gas solution

Spray FReezing into Liquid (SFL) Technology

The Dow Chemical Company of Midland, Michigan, commercialized a process pioneered at the University of Texas at Austin in Austin, Texas. A feed solution including an API and dissolution enhancing excipient(s) is atomized directly into a cryogenic liquid, such as nitrogen, in this particle engineering technology. The active pharmaceutical ingredient (API) is molecularly dispersed with a polymer in a matrix to form the final dry powder's discrete microparticles. The SFL method resulted in nanoparticles of danazol that were both extremely effective and protected by larger structured aggregates. The SFL powders have much quicker dissolving rates. Micronized bulk danazol had a slow dissolving rate, with only 30% dissolving in 2 minutes. SFL danazol/PVP K-15 powders with high surface areas and glass transition temperatures were found to stay amorphous and to dissolve quickly even after 6 months of storage, according to a recent study. [26] The EPAS method was initially developed at UT Austin and then brought to market by Dow Chemical company. In the Evaporative Precipitation into Aqueous Solution (EPAS) method, the active pharmaceutical ingredient (API) precipitates when an organic solvent in the feed solution evaporates when the two solutions are heated to near or above boiling point. Fast evaporation of the feed solvent causes fast saturation of the aqueous solution, super saturation, nucleation, and precipitation of the dissolved API with the dissolution-enhancing excipients. The dissolution of danazol (2%) and stabilizer (0-1%; povidone K15, poloxamer 407) was achieved by dissolving them in dichloromethane. The resulting solution was then pumped through a preheating coil using a high-performance liquid chromatography (HPLC) pump. Subsequently, the solution was sprayed into a heated receiving vessel, where a stabilizer (0-2%; e.g., povidone K-15, deoxycholic acid) dissolved in water at 80°C, using a fine nozzle. (The crimped tube, with or without an ultrasonic horn, has an inner diameter of 127 microns.) The solution spray produced a narrow stream of tiny droplets that evaporated quickly, resulting in the precipitation of danazol through either nozzle. The results of the investigations on EPAS processed danazol formulations indicate that these formulations demonstrated much greater rates of dissolution, with more than 90% of the drug dissolving within a 10-minute timeframe. Additionally, these formulations exhibited high surface areas, exceeding 40 m2/g, and low levels of crystallinity when compared to the bulk danazol or physical combination.[27]

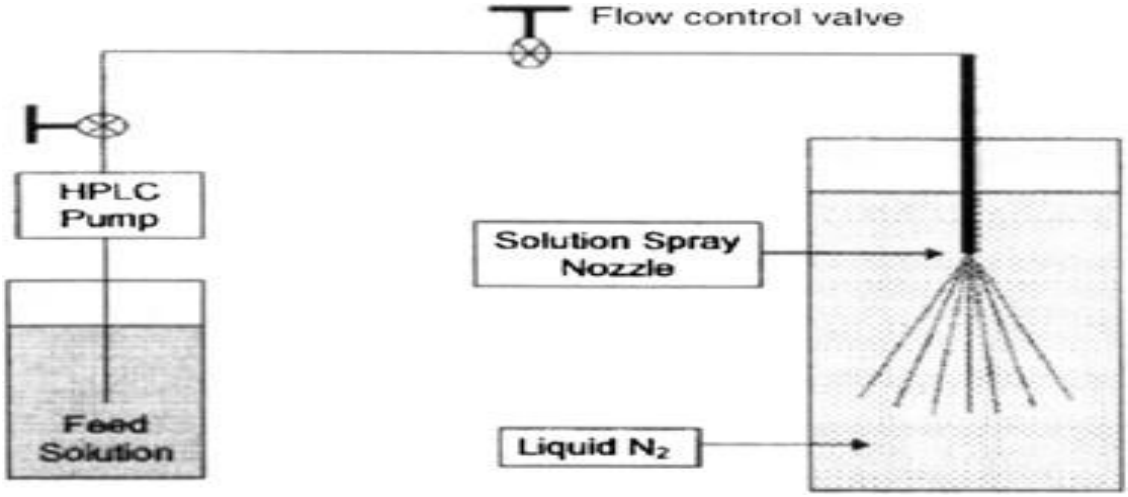

Fig (5). Spray freezing into liquid (SFL) technology

SELECTION OF STABILIZER FOR NANOCRYS

The selection of stabilizers is of critical importance.The selection of stabilizers plays a crucial role in nanocrystal formulations as it directly influences the final particle size through the type and concentration of the stabilizer employed. Stabilizers additionally serve the purpose of inhibiting the aggregation of nanocrystals. Polymers or surface-active compounds function by enveloping the surface of drug nanocrystals, so creating a steric barrier that imparts stability. The significance of dispersion or van der Waals forces in particle interactions becomes pronounced at the nanoscale in nanocrystal formulations, as the type and concentration of the stabilizer play a crucial role in determining the final particle size. Stabilizers additionally serve the purpose of inhibiting the aggregation of nanocrystals. Polymers or surface-active chemicals function by encapsulating medication nanocrystals, thereby creating a steric barrier that imparts stability to the system. The significance of interparticle forces, namely dispersion or van der Waals forces, becomes pronounced when considering phenomena occurring at the nanoscale. Surface free energy (ΔG) is high in nanosized particles having a large surface area. As a result, particles tend to agglomerate in order to reduce surface free energy, resulting in an increase in particle size and a decrease in surface area. As a result, a stabilizer reduces ΔG by decreasing the interfacial tension. [28]

$$\Delta G = \Delta A.\frac{\gamma s}{I}$$

$\frac{\gamma s}{I}$: Interfacial tension between the surface of solid and the surrounding liquid phase (joule/m2)

ΔG: change in surface free energy (joule)

ΔA: change in the surface area (m2)

stabilizer is an important factor as its concentration that affects the physical stability of final product [29] The following polymers are acceptable for use as stabilizers: hydroxypropyl cellulose (HPC), hydroxypropyl methyl cellulose (HPMC), povidone (PVP K30), and pluronics (F68 and F127). In order to provide a steric layer, the chains should be long enough, but not too long as to slow down dissolution. An example of a surfactant stabilizer that is suitable for physical stability is sodium lauryl sulfate anionic and polysorbate 80 (nonionic). Furthermore, because drugs particles are typically quite hydrophobic, surfactants frequently aid in wetting, electrostatic stability, and dispersion of the particles. In the formulation of drug nanocrystals that are currently available on the market, stabilizers such HPMC E3, Povidone, and SLS have been employed. [30]

NANOCRYSTAL STABILIZERS

Despite the many remarkable benefits of nanocrystals, their small size frequently raises stability issues. Nanocrystals' large surface area produce sufficiently high free energies or surface charges that could lead to attraction or agglomeration. [31] The process of recrystallizing into larger

particles, also known as Ostwald ripening, is promoted when small-sized nanocrystals raise the solubility of a drug beyond its saturation point. These processes eventually result in irreversible loss of formulation integrity [32] A variety of stabilizers are employed to keep Nanocrystals stable.

Poloxamer

Poloxamers are amphiphilic block copolymers that feature an EPE configuration of hydrophilic (ethylene oxide) and hydrophobic (propylene oxide) units. The length of the polymer blocks used to create the various grades of poloxamers. In addition to their usefulness as stabilizers, they may help reduce the chemoresistance of MDR cells. Poloxamers are appropriate for intravenous medication delivery because they are a GRAS excipient with a reduced potential for hemolytic responses. In a 2014 study. They have seen widespread application in the stabilization of nanocrystals. Due to compound shielding, omeprazole nanocrystals with a poloxamer 188 attachment were more stable than omeprazole solution. Poloxamer 407 was also utilized by scientists in the creation of several nanocrystal compositions. Deng tried to make paclitaxel more effective as a treatment by stabilizing its nanocrystals with poloxamer 407, but this only resulted in a micellar structure that melted at room temperature. Long-lasting nanocrystals were formed using renanonization via incubation sonication [33]

Polyvinyl Pyrrolidone (PVP)

Acetylene and pyrrolidone react to yield vinyl pyrrolidone, which is then polymerized into PVP (also known as povidone). It can be used as a binder in tablets and capsules, a film forming in ophthalmic solutions, a taste masking agent, a toxicity reduction, and most critically, as a suspension stabilizer, and it comes in a variety of viscosity grades. For more information, see [34] Celecoxib nanocrystals were successfully synthesized with the help of the stabilizer PVP K30. DSC analysis showed that the drug's crystallinity was unaffected by the stabilizer combination; nonetheless, melting point decreases were found due to the creation of new crystalline states [35] Probucol nanocrystals were made using PVP K17 and K12, proving the PVP's usefulness in a wide variety of contexts. The results showed that either PVP or SDS alone couldn't stop clumping, but that the two together produced a stable formulation. [36]

Polyvinyl Alcohol (PVA)

The level of polymerization and the degree of hydrolysis affect the water soluble PVA's properties. PVA that has been partially hydrolyzed is typically employed in the pharmaceutical sector. [37] It has been utilized to create stable nitrendipine (a class II calcium channel blocker) nanocrystals by a precipitation ultra-sonication process, resulting in better dissolving properties and greater oral bioavailability. [38]

Lecithin

Lecithin is a complex lipid that includes a wide variety of fatty acids, carbohydrates, and phosphatides. Their high lipid concentration makes them a vital part of a wide variety of dietary supplements. The pharmaceutical sector makes excellent use of them as stabilizers and emulsifiers. Their high absorption enhancing characteristics are based on the free fatty acid concentration, therefore their physical forms can range from powders to semi-liquids. Egg and soy are two common examples of natural materials that be processed into drug stabilizers. Amoitone B, an anticancer agent, was stabilized by combining lecithin with Poloxamer 188 and HPMC. The bioavailability of nebulized itraconazole nanocrystals was improved by the use of dipalmitoyl phosphatidylcholine (a lecithin; a natural component of human lung surfactant) by Yang et al. Itraconazole's overall in-vivo presence was enhanced thanks to the permeability-promoting effects of dipalmitoyl phosphatidylcholine.

Brij

Brij-78, a nonionic surfactant also known as Cremophor contains Polyoxymethylene alkyl ethers, is utilized as an emulsifying, wetting, and permeation-enhancing agent. Gao et al. used Brij-78 to stabilize the nanocrystals they produced by processing oridonin. Its use is, however, frequently associated with peripheral neuropathy, anaphylactic hypersensitivity reactions, hyperlipidemia, aberrant lipoprotein patterns, erythrocyte aggregation, and hyperlipidemia. [39]

Polysorbate 80

A significant pharmaceutical excipient known as polyoxymethylene sorbitol fatty acid ester derivative has emerged. They are categorized based on the kind of fatty acid moiety which impacts their actions. Although it has been widely employed as a surface active agent, its propensity to cause hypersensitivity, child birth weight loss, and other adverse consequences when used in high concentrations can occasionally function as a deterrent. As a result, researchers frequently use Polysorbate 80 as an additional stabilizer at low concentrations. It has been used to produce fenofibrate nanocrystals when combined with poloxamer 188, PVA, PVP, and SDS. [40]

HPMC

It is a GRAS-listed pharmaceutical excipient that has proven useful in the formation of nanocrystals and is therefore widely utilized. It is believed that this will completely cover the crystal surface, providing sufficient stability. Since its melting point is higher than that of most stabilizers, it is a safe bet for use in high-temperature industrial processes. Ali et al., in a recent study, used HPMC to stabilize hydrocortisone nanocrystals during the medium milling process for ocular injection. HPMC played a vital role in stabilizing the nanocrystals by completely covering the dried particle's surface and imparting a low zeta potential. Nanocrystals of the BCS lass-II medicines fenofibrate, naproxen, and griseofulvin were synthesized by Figueroa et al. using HPMC. HPMC was inferred to have preserved the bioactive's crystalline structure. Nimodipine nanocrystals were produced by Hecq et al. using HPMC. We employ high-pressure homogenization. The process was used to generate nanocrystals of nifedipine, which dissolves far faster than the original drug. When compared to other surface-active agents like SDS, poloxamer, and polysorbate, the stabilizing effect of low-viscosity grade HPMC was satisfactory. According to the research [41]

DOSAGE FORM DEVELOPMENTS OF NANOCRYSTALS

Tablets, capsules, pellets, and injectable solutions are common post-nanonization dosage forms for drug nanocrystals. Incorporating drug nanocrystals into new dosage forms necessitates removing the suspension solvent without altering the drug's physical, chemical, or pharmacological properties. Drug nanoparticles have been dried or concentrated using numerous processes, including freeze drying, spray drying, centrifugation, and ultrafiltration. In order to prevent the nanoparticles from clumping together, stabilizers including mannitol, sucrose, and trehalose are typically added to the mixture. The freeze-drying process relies heavily on the type and quantity of protectants used, as well as the freezing rate. To ensure that the characteristics of drug nanoparticles are maintained in solid dosage forms, it is necessary to add excipients such as fillers, binders, humectants, disintegration agents, and lubricants. [42]

MARKETED PRODUCTS BASED ON NANOCRYSTAL TECHNOLOGY

Rapamune

The first FDA-approved oral nanocrystals were introduced by Wyeth Pharmaceuticals (Madison, NJ) in the year 2000. Composed of Sirolimus nanocrystals and an excipient blend, the resulting compound is amenable to direct compression into ingestible tablets. The nanocrystal tablets have a 21% greater oral bioavailability than Sirolimus solution. Once upon a time, Rapamune was available only as a refrigerated oral solution that was to be diluted with water and orange juice prior to administration [43]

Emend

Merck (Winehouse Station, NJ) initially released it to the public in 2001. Aprepitant, an effective treatment for nausea and vomiting, is included. Aprepitant selectively and potently affinizes human substance P/neurokinin 1 (NK 1) receptors. Serotonin (5-HT 3), dopamine, and corticosteroid receptors are the focus of current treatments for chemotherapy-induced nausea and vomiting (CINV). There is almost no affinity between aprepitant and these receptors. In animal studies, aprepitant's central effects mitigated the nausea and vomiting caused by cytotoxic chemotherapy medicines like cisplatin. Drug nanocrystals of aprepitant 80 or 125 mg are contained in each capsule of Emend. It was published in 2007 [44]

Tricor

Fenofibrate, available in the form of tablets with a dose of 48 mg and 145 mg, is the active ingredient, and Abbott Laboratories introduced it to the market. In adult patients with primary hypercholesterolemia or mixed dyslipidemia (Fredrickson types IIa and IIb), Tricor is recommended as an adjunctive therapy to diet in order to increase HDL-C (high-density lipoprotein cholesterol), lower triglycerides (TG), lower LDL-C (low-density lipoprotein cholesterol), lower total cholesterol (Total-C), and lower Apo lipoprotein B (Apo B) [45]

Megace ES

Par Pharmaceutical Companies, Inc. (Spring Valley, NY) developed Megace Es (megestrol acetate), and Bristol-Myers Squibb (New York) licensed the Megace trade name. Like natural progesterone, megestrol is a synthetic progestin. Megestrol also suppresses pituitary luteinizing hormone release and has direct lethal effects on breast cancer cells in tissue culture. Patients undergoing chemotherapy or those infected with HIV use it to increase their weight and appetite [46]

DRUGS IN THE PIPELINE

In addition to the above-mentioned commercialized products, numerous other nanocrystal-based medications are still undergoing clinical trials.

Nanotax® (NT)

NT is a nanocrystal suspension in water of pure paclitaxel; no other excipients such Cremophor EL, alcohol, or albumin have been added. In late 2008, Criti Tech, USA initiated a Phase I clinical trial for cancer treatment. Fast nucleation (continuous supercritical fluid process) results in NT with a low surface charge, which allows the particles to remain suspended for months at a time. Administered intraperitoneally, it has a decreased frequency of adverse effects, leading to more patient compliance. [47]

Zolip®

Zolip®, developed by Solvay Pharmaceuticals of Belgium, combines 145 milligrams of fenofibrate with 40 milligrams of simvastatin to treat mixed hyperlipidemia (cardiovascular disorders). Improvements in efficacy and safety have been observed in phase III studies. [48]

TMC278

In July of 2010, Tibotec, a Belgian company, introduced TMC278 (nanocrystals of rilipivrin) to the US Food and Drug Administration as a non-nucleoside reverse transcriptase inhibitor using Elan nanocrystal technology. This stable, long-acting parentral suspension may make HIV treatment more accessible, both for maintenance and prevention[49]

TheraluxTM (TL)

Thymectacin nanocrystals, marketed as TheraluxTM, were first made available to people with non-Hodgkin's lymphoma by the Canadian company Celmed Biosciences. Clincal trails for TL’s

use of photodynamic therapy, a non-invasive procedure is currently in their Second phase. Tl has been found in studies to be highly effective at eradicating cancer cells without effecting healthy ones. [50]

Clozapine (CP)

Using Elan nanocrystal technology, Azur Pharma of Ireland has developed a once-daily CP tablet with extraordinarily high absorption. Clinical trials are underway. It is indicated for the management of severely ill schizo-phrenic patients who fail to respond adequately to standarddrug treatments for schizophrenia [51]

APPLICATION OF NANOCRYSTALS

Oral Drug Delivery

The oral route of administration is widely regarded as the most secure and optimal method of drug administration. [52] Dissolution is thought to be an absorption rate determining stage for medications that are taken orally. Increased drug absorption is a result of the faster dissolution rate made possible by nanocrystals, which in turn raises the saturation solubility. By embedding nanocrystals in a solid PEG matrix, Muller et al. have improved upon oral administration of thermostable medicines using melted PEG (melting point at 60 °C). To make the tablet or capsule, the nanocrystal suspension was milled to a powder and then filled into capsule shell. Therefore, this innovative drug delivery technology provides a means to increase the oral bioavailability of poorly soluble medications by incorporating them directly into tablet, capsule, or hot melts solid matrix. [53]

Intravenous Drug Delivery

When it comes to overcoming the negative effects of i.v. formulations caused by poorly tolerated excipients (such Cremophor EL in Taxol® and cyclodextrin in Sporanox®), aqueous nanosuspensions are the way to go. However, the pharmacokinetics of injecting nanosuspensions are distinct from those of injecting drug solutions. Not dissolving quickly enough, nanocrystals larger than 200 nm are taken up by liver macrophages, where they may cause liver damage. Injecting nanosuspensions of nanocrystals so small that their size is considerably below 100 nm causes them to disintegrate significantly more quickly. Therefore, they are thought to be more suitable for imitating solutions administered intravenously and minimizing or avoiding uptake by the liver. [54]

Pulmonary Drug Delivery

In the case of poorly soluble medications, nanosuspensions can be utilized as an alternative to dry powders for inhalation. Aqueous nanosuspensions can be applied by simply adding them to an aqueous nebulizer. The nebulizer produces an aerosol with droplet sizes that are appropriate for pulmonary delivery, such as 1 to 5 micrometers. These droplets hold the nanocrystals in place. As a powder, the nanocrystals cannot be inhaled. In addition to being very sticky and having a propensity to aggregate, nanocrystals also exhale particles that are between 0.5 and 1 m in size. Nanocrystals have the advantage of having a faster rate of disintegration than micronized crystals. Particularly when stabilized with surfactants with strong spreadability, aerosol droplets should spread more evenly on the lung surface when they deposit in the lung as tiny particles. Budesonide (corticosteroid) nanosuspensions have been effectively created for pulmonary administration. In-depth research was done on the transport of nanosuspensions to the lungs [55] by comparing different commercial portable nebulizers regarding their ability to nebulize nanosuspensions. [56] All of them were suitable, showing that nebulization of well-stabilized nanosuspensions can be performed successfully.

Ocular Drug Delivery

This potential use of nanosuspensions as a medicine delivery method has not yet been explored. Unlike solutions, which are quickly flushed out of the eye, nanoparticle suspensions with sticky

characteristics can demonstrate sustained release. Compared to currently available commercial formulations, polymeric (Eudragit RS 100 and Eudragit RL 100) nanoparticulate suspensions of flurbiprofen and ibuprofen demonstrated superior in vivo performance and were able to maintain drug release for 24 hours. Benefiting from a longer period spent in a cul-de-sac, nanosuspensions can also be used for medications that show low solubility in lachrymal fluids. There is a dearth of research into the use of NSAID nanocrystals for ocular purposes. Ocular nanosuspensions of hydrocortisone, prednisolone, and dexamethasone have been observed to increase the pace of drug absorption, the extent of drug absorption, and the intensity of therapeutic action. The regulatory advantages of nanosuspensions over polymeric nanoparticles are striking. The particles travel into the nasal cavity and then the throat via the lipophilic channels. Therefore, formulation components must be safe for topical application to the eye. Considering the fact that many polymers are not approved by the official authoritirs Because nanosuspensions are made up entirely of the drug and a very little quantity of stabilizer, they do not contain any matrix material. [57]

Dermal Drug Delivery

Cosmetic and pharmaceutical actives that aren't easily absorbed by the skin can be better delivered with the help of nanocrystals. Because of the larger concentration gradient created by the higher saturation solubility, passive diffusion is facilitated. When molecules enter the skin, they are quickly replaced by others that dissolve from a nanocrystal depot in the cream. Juvena introduced the world's first four cosmetic nanocrystal products containing rutin. Bioactivity (as determined by SPF) of the original rutin molecule in nanocrystal formulation is 500 times higher than that of the water-soluble rutin glucoside. [58] Of course, the same idea can be applied to other pharmaceuticals that are dermally relevant yet have limited solubility

Ophthalmic Drug Delivery

The extended duration of nanoparticle retention in the eye is likely attributed to their sticky characteristics. Nanosuspensions could potentially serve as a viable means of administering medications with low solubility. The objective of developing colloidal delivery systems for ophthalmic applications is to provide dropable dosage forms that possess a substantial drug loading capacity and provide prolonged medication efficacy. The nanosuspensions were generated by a modified version of the quasi-emulsion solvent diffusion method, employing different formulation parameters such as the drug-to-polymer ratio, total amount of drug and polymer, and stirring speed. Nanosuspensions exhibited an average size of approximately 100 nm and possessed a positive charge, as shown by a zeta-potential ranging from +40 to +60 mV. These characteristics render them well-suited for utilization in ocular applications. Stability assessments, encompassing storage durations of up to 24 months at a temperature of 4 degrees Celsius or at ambient conditions, were conducted in order to develop an appropriate pharmaceutical formulation. The results of in vitro dissolving studies demonstrated that nanoparticles exhibited a regulated release profile of IBU. The efficacy of the treatment was evaluated in vivo using a rabbit model.

Targeted Drug Delivery

Since the surface properties of nanoparticles and the stabilizing agent may be easily modified, they can be employed for targeted delivery. The creation of economically feasible nanosuspensions for targeted drug delivery is made possible by their adaptability, scalability, and commercial producibility. When macrophages are not the intended targets, the natural targeting mechanism may present challenges. Therefore, its surface potential must be modified to avoid phagocytic uptake. Kayser created a nanosuspension of aphidicolin to enhance the drug's targeted efficacy against macrophages infected with Leishmania. According to his research, [58] aphidicolin is quite effective at microgram concentrations. The use of nanosuspensions allows for the safe administration of medications with low solubility to the brain. Microparticulate busulfan injected

intrathecally in mice has been linked to significant efficacy. Intrathecally injected surface-modified polyisobutyl cyanoacrylate nanoparticles have been used successfully to deliver the peptide Dalargin to the brain. Also, using surface modified polyisobutyl cyanoacrylate nanoparticles, the peptide Dalargin was successfully delivered to the brain.

CONCLUSION

For formulation scientists attempting oral administration of therapeutic molecules, poor water solubility is quickly becoming the primary obstacle, necessitating the use of innovative formulation strategies. Drug nanocrystals are a universal formulation strategy for improving the therapeutic efficacy of these medications regardless of administration method. Any medication can be scaled down to the nanoscale range. Drug nanocrystals are no longer the last resort because of the considerable flexibility with which they can be manufactured.

REFERENCE

1. Life sciences new brief http://www.biopharm.org.tw/ic/canada strategies/05052004/eindex.html (Accessed May 15, 2010). Lipinski C, Poor aqueous solubility— an industry wide problem in drug discovery, Am.Pharm. Rev.2002; 5: 82–85
2. Keck C, Kobierski S, Mauludin R, Müller RH, Second generation of drug nanocrystals for delivery of poorly soluble drugs: smart crystals technology,D O S I S,2008; 24(2):125-127.
3. Troester F, Cremophor-free aqueous paclitaxel nanosuspension—production and chemical stability, Controlled Release Society 31st annual meeting, Honolulu, HI, 2004.
4. Möschwitzer J, Achleitner G, Pomper H, Muller RH, Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology, European Journal of Pharmaceutics and Biopharmaceutics, 2004; 58(3):615–619.
5. Noyes,W. Whitney, The rate of solution of solid substances in their own solutions, J. Am. Chem. Soc. 19 (1897) 930–934.
6. V.B. Patravale, A.A. Date, R.M. Kulkarni, Nanosuspensions: a promising drug delivery strategy, J. Pharm. Pharmacol. 56 (7) (2004) 827–840.
7. Y. Wu, A. Loper, E. Landis, L. Hettrick, L. Novak, K. Lynn, C. Chen, K. Thompson, R. Higgins, U. Batra, S. Shelukar, G. Kwei, D. Storey, The role of biopharmaceutics in thedevelopment of a clinical nanoparticle formulation of MK-0869: a beagle dog model predicts.improved bioavailability and diminished food effect on absorption in human, Int. J. Pharm.285 (1– 2) (2004) 135–146.
8. M. Mosharraf, C. Nystrom, The effect of particle size and shape on the surface specific dissolution rate of micronized practically insoluble drugs, Int. J. Pharm. 122 (1995) 35– 47.
9. R.H. Muller, K. Peters, Nanosuspensions for the formulation of poorly soluble drugs I. Preparation by a size-reduction technique, Int. J. Pharm. 160 (1998) 229–237
10. Buchmann S, Fischli W, Thiel FP, Alex R. Aqueous microsuspension, an alternative intravenous formulation for animal studies. In: 42nd annual congress of the International Association for Pharmaceutical Technology (APV), Mainz, 1996.
11. Bruno JAD, Brian D. Gustow Evan, Illig, Kathleen J, Rajagopalan, Nats, Sarpotdar. Method of grinding pharmaceutical substances. US 5,518,187, 1992.
12. Wyeth Research Drug Information, Rapamune (Sirolumus) Oral Solutions and Tablets. Company Communications, 2004.
13. Merck, Drug Information Emend. 2004.
14. Speiser PP (1998) Poorly soluble drugs, a challenge in drug delivery, in Muller RH, Benita S and Bohm B (eds.), Emulsions and Nanosuspensions for the Formulation of Poorly Soluble Drugs, Medpharm Scientific Publishers: Stuttgart.
15. Bruno RPM (1999) Microfluidizer Processor Technology for High Performance Particle Size Reduction, Mixing and Dispersion. Microfluidizer Processor Technology.

16. Parikh IS, Ulagaraj Composition and method of preparing microparticles of waterinsoluble substances. 93969997, 1997
17. Haynes DH. Phospholipid-coated microcrystals: injectable formulations of waterinsoluble drugs. US 5,091,187,1992
18. Miiller RH, Jacobs C and Kayser O (2000) Nanosuspensions for the formulation of poorly soluble drugs, in Nielloud F and Marti-Mestres G (eds.) Pharmaceutical Emulsions and Suspensions, Marcel Dekker
19. Muller RH, Jacobs C and Kayser O (2003) DissoCubes — A novel formulation for poorly soluble and poorly bioavailable drugs, in Rathbone MJ, Hadgraft}, Roberts MS (eds.), Modified- Release Drug Delivery Systems, Marcel Dekker.
20. Radke M, Pure drug nanoparticles for formulation of poorly soluble drug, New drug delivery: www.pharmasol-berlin.de. Kipp JE, Tak Wong JC, Doty MJ, Rebbeck CL. US Pat. application no. 20020168402 A1.2002
21. Türk M, Hils P, Helfgen B, Schaber K, Martin HJ, Wahl MA, Micronization of pharmaceutical substances by Rapid Expansion of Supercritical Solutions (RESS): a promising method to improve the bioavailability of poorly soluble pharmaceutical agents, J Supercrit Fluids,2002:75–84.
22. Katteboinaal S, Chandrasekhar P, Balaji S, Drug nanocrystals: a novel formulation approach for poorly soluble drugs, International Journal of PharmTech Research, 2009; 1(3): 682-694.
23. Li Zhiyi, Preparation of griseofulvin microparticles by supercritical fluid expansion depressurization processPowderTechnology Volume 182, Issue 3, 10 March 2008, Pages 459-465.
24. Andrian Tandya, Micronization of cyclosporine using dense gas techniques, The Journal of SupercriticalFluids, Volume 37, Issue 3, May 2006, Pages 272-278.
25. Young, Phospholipid-Stabilized Nanoparticles of Cyclosporine A by Rapid Expansion from Supercritical to Aqueous Solution AAPS PharmSciTech 2003; 5 (1) Article 11.
26. Physical stability of micronized powders produced by spray-freezing into liquid (SFL) to enhance the dissolution of an insoluble drug. Rogers TL, Johnston KP, Williams RO 3rd. Pharm Dev Technol. 2003;8(2):187-97.
27. Böhm B. Production and characterisation of nanosuspensions as novel delivery system for drugs with low bioavailability. In: Pharmaceutical Technology. Berlin: Free University of Berlin, 1999.
28. Merisko-Liversidge E, Liversidge GG, Cooper ER, Nanosizing: A Formulation Approach for Poorly-Water-Soluble Compounds, Eur J PharmSci.2003; 18: 113-120.
29. Kesisoglou F, Panmai S, Wu Y, Nanosizing– Oral formulation development and biopharmaceutical evaluation, Adv Drug DelivRev,2007;59: 631–644.
30. Gao L., Zhang D. et al, Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system, J Nanopart Res.2008; 10: 845- 862.
31. Vishal V. Pande and Vidya N. Abhale Nanocrystal technology: A particle engineering formulation strategy for the poorly water soluble drugs Scholars Research Library 2016; 8 (5); 384-392.
32. Y. Wang, Y. Zheng, L. Zhang, Q. Wang, D. Zhang, Stability of nanosuspensions in drug delivery, J. Control. Release 172 (2013); 1126-1141.
33. J. Deng, L. Huang, F. Liu, Understanding the structure and stability of paclitaxel Nanocrystals, Int. J. Pharm. 390 (2010); 242-249.
34. Vivek K. Pawar, Yuvraj Singh, Jaya Gopal Meher, Siddharth Gupta, Manish K. Chourasia Engineered nanocrystal technology: In-vivo fate, targeting and applications in drug delivery, Journal of Controlled Release 183:(2014):51-66.
35. Pongpeerapat, C. Wanawongthai, Y. Tozuka, K. Moribe, K. Yamamoto, Formation mechanism of colloidal nanoparticles obtained from probucol/PVP/SDS ternary ground mixture, Int. J. Pharm. 352; (2008); 309-316.

36. Dolenc, J. Kristl ,S.Baumgartner,O. Planinsek, Advantages of celecoxib Nanosuspension formulation and transformation into tablets, Int. J. Pharm. 376 (2009);204- 212.
37. D. Douroumis, A. Fahr, Stable carbamazepine colloidal systems using the cosolvent technique, Eur. J. Pharm. Sci. 30; (2007); 367-374.
38. R.C. Rowe, P.J. Sheskey, S.C. Owen, Handbook of Pharmaceutical Excipients,Pharmaceutical Press, London, 2006
39. J. Lee, S.-J. Lee, J.-Y. Choi, J.Y. Yoo, C.-H. Ahn, Amphiphilic amino acid copolymers asstabilizers for the preparation of nanocrystal dispersion, Eur. J. Pharm. Sci. 24(2005); 441-449
40. L. Gao, D. Zhang,M. Chen, T. Zheng, S.Wang, Preparation and characterization of anoridoninnanosuspension for solubility and dissolution velocity enhancement,Drug Dev. Ind. Pharm. 33; (2007); 1332- 1339.
41. Ghosh, S. Bose, R. Vippagunta, F. Harmon, Nanosuspension for improving the bioavailability of a poorly soluble drug and screening of stabilizing agents to inhibitcrystal growth, Int. J. Pharm. 409; (2011); 260-268.
42. Chen H, Khemtong C, Yang X, Chang X and Gao J, Nanonization strategies for poorly water soluble drugs, Drug Discovery Today, 2010;00:1-6.
43. A.H. Shojaei, Buccal mucosa as a route for systemic drug delivery: a review, J.Pharm. Pharm. Sci. 1 (1998); 15±30.
44. Hanafy A, Spahn H, Vergnaut G, Grenier p, GrozdanisMT,Lenhardt T, Pharmacokinetic evaluation of oral fenofibrate and nonosuspension and SLN in comparision with conventional suspension of micronized drug. Adv Drug Del Rev 2007;19-26.
45. Kipp JE. The role of solid nanoparticle technology in the parenteral delivery of poorly water-soluble drugs. Int J Pharm2004;284(1-2);109-122.
46. Jens-Uwe A H Junghanns and Rainer H Müller Nanocrystal technology, drug delivery and clinical applications Int J Nanomedicine. 2008 Sep; 3(3): 295±310.
47. Nanotax clinical trial. Kansas cancer treatmenthttp://www.kansascancertreatment.org/ (Accessed May 8, 2010).
48. Zolip 0502: Comparison of the combination of fenofibrate and 40mg simvastatin versus 40 mg simvastatin monotherapy; Solvay Pharmaceuticals. http://www.druglib.com/trial/83/NCT00352183 html (Accessed May 15, 2010).
49. Tibotec Pharmaceuticals Submits New Drug Application for Investigational Once Daily HIV Treatment TMC278 to U.S. Food and Drug Administration. http://www.jnj.com/connect/news/product/ Tibotec-Pharmaceuticals-Submits-New-Drug-Application-for-Investigational-Once-Daily-HIV-Treatment-TMC278-to-US-Foodand-Drug-Administration (Accessed December 10, 210).
50. Life sciences news brief. http://www.biopharm.org.tw/ ic/ Canada/strategies (Accessed May 15, 2010).
51. Nanotechnology may deliver once-daily dosage of clozapine. Drug discovery & development. once-daily dosage of clozapine. Drug http://www.dddmag.com/news-once-daily-dosing-Clozapine.aspx (Accessed November 30, 2010).
52. J.-J. Guo, P.-F. Yue, J.-l. Lv, J. Han, S.-S. Fu, S.-X. Jin, S.-Y. Jin, H.-L. Yuan, Developmentand in vivo/in vitro evaluation of novel herpetrione nanosuspension, Int. J. Pharm.441 (2013); 227±233.
53. Femia R. Megestrol acetate nanocrystal: Results of doseescalating studies under fed and fasting conditions. California, USA. 2005.
54. Müller RH and Jacobs C, Buparvaquone mucoadhesive nanosuspension: preparation, optimisation and long-term stability, Int. J. Pharm, 2002; 237: 151–161.
55. Hernandez-TrejoN, Kayser O, Steckel H and Muller RH, Characterization nebulized buparvaquone nanosuspensions effect of nebulization technology, J. Drug Target, 2005; 13: 499–507.
56. Bucolo C, Maltese A, Puglisi G and Pignatello R, Enhanced ocular anti-inflammatory activity

of ibuprofen carried by an Eudragit RS100 nanoparticle suspension, Ophthalmic Res,2002; 34 : 319–323.
57. Katteboinaal S, Chandrasekhar P, Balaji S, Drug nanocrystals: a novel formulation approach for poorly soluble drugs, International Journal of PharmTech Research, 2009; 1(3): 682-694.
58. Kayser O, Nanosuspensions for the formulation of aphidicolin to improve drug targeting effects against Leishmania infected macrophages, International Journal of Pharmaceutics, 2000;196(2):253-25