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NANOCRYSTAL TECHNOLOGY AND DRUG DELIVERY

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ABSTRACT

Products of nanotechnology are expected to revolutionize modern medicine, as evidenced by recent scientific advances and global initiatives to support nanotechnology and nanomedicine research. The field of drug delivery is a direct beneficiary of these advancements. Poor aqueous solubility is clearly recognized by the pharmaceutical industry as a major problem. The great challenge for the pharmaceutical development is to create new formulation approaches and drug-delivery systems to overcome solubility problems of these drug candidates which are also often associated with poor oral bioavailability. The nanocrystal technology will continue to thrive as a useful tool in pharmaceutics for the improvement of drug solubility, oral absorption, and hence, bioavailability. Almost any drug can be reduced in size to the nanometer range. The novel and potential applications of nanotechnology in pharmaceutics are; development of diagnostic tools, formulation of drug carrier systems and gene therapy.

Keywords: Nanocrystal, poorly soluble drugs, top down, bottom up.

INTRODUCTION

Nanocrystal is described as any nanomaterial with at least one dimension ≤ 100nm and that is single crystalline. (1) The term “drug nanocrystals” implies a crystalline state of the discrete particles, but depending on the production method they can also be partially or completely amorphous. A nanosuspension consists of drug nanocrystals, stabilizing agents such as surfactants and/or polymeric stabilizers, and a liquid dispersion medium. The dispersion media can be water, aqueous solutions, or nonaqueous media. Drug nanocrystals have to be distinguished from polymeric nanoparticles, which consist of a polymeric matrix and an incorporated drug. Drug nanocrystals do not consist of any matrix material.

During the last two decades, many modern technologies have been established in the pharmaceutical research and development area. The automation of the drug discovery process by technologies such as high-throughput screening, combinatorial chemistry, and computer-aided drug design is leading to a vast number of drug candidates possessing a very good efficacy. Unfortunately, many of these drug candidates are exhibiting poor aqueous solubility. Long before one of these compounds can reach the market; it needs to be formulated for the pharmacological activity tests and for the preclinical studies. The great challenge for the pharmaceutical development is to create new formulation approaches and drug-delivery systems to overcome solubility problems of these drug candidates which are also often associated with poor oral bioavailability (2, 3).

Nanotechnology is derived from the Latin word “nano”, which means dwarf. It is used in the production of materials at submicron or molecular level in engineering, electronics, physics and material science. Nanosized materials may be a device, a system of supramolecular chemistry, complexes or compounds. In 1959, the term of nanotechnology was first used by the physicist Richard Feynman (4). He indicated that by producing materials and devices at molecular level, nanostructures can be measured and nanotechnology can be used for many new purposes.

The major application areas of nanotechnology are

1. materials and manufacturing sector,
2. nano electronics,
3. computer technology (fiber optic communications networks),
4. aviation and space research,
5. environment and energy,
6. agriculture,
7. chemical engineering,
8. defense industry,
9. biology, biotechnology,
10. Medicine and pharmaceutics.

In medicine and pharmaceutics, nanotechnology is used to improve human health at a molecular level. The novel and potential applications of nanotechnology in pharmaceutics are; development of diagnostic tools, formulation of drug carrier systems and gene therapy (5). The advantages of nanotech drugs compared to conventional counterparts lie on the basis

of particle size. Drugs/drug products with nano dimension can be used at a lower concentration and can lead to early onset of bioactivity (6). Nano drug delivery systems (nanopharmaceutics) are, but not limited to, nanocapsules, nanospheres, nanosponges, nanoemulsions, solid lipid nanoparticles, nanovesicular systems (liposomes, niosomes), molecular systems (inclusion complexes) and nanocrystals.

The dissolution rate of drugs in the GI tract affects absorption rate and degree of drugs. Absorption of a drug is defined as the transition of a drug from the applied place to the blood and/or lymphatic circulation. The dissolution velocity (low solubility in general is correlated with low dissolution velocity, law by Noyes-Whitney) and intestinal permeability are key determinants for the bioavailability, particularly for per orally administered drugs. To evaluate and characterize pharmaceutical compounds with respect to their aqueous solubility and intestinal permeability, a biopharmaceutics classification system has been developed (7, 8).

The Biopharmaceutics Classification System		
Class	**Solubility**	**Permeability**
I	High	High
II	Low	High
III	High	Low
IV	Low	Low

A solubility enhancement cannot necessarily solve the bioavailability problems of class IV drugs in any case. Drug candidates for a successful improvement of their bioavailability by a solubilization technique belong to class II which means that their bioavailability is only limited by their poor aqueous solubility/dissolution velocity. The term “solubilization techniques” in the present context means technologies which increase the dissolution velocity dc/dt and—ideally—also the saturation solubility c_s

There are many conventional approaches for the solubilization of poorly soluble drugs.

- Salt formation and pH adjustment are the first attempts if the molecule is ionizable, because in general the ionized species has a higher aqueous solubility compared to the neutral one.

- Co solvents such as propylene glycol are used, especially for parenteral or liquid oral dosage forms. Systemic toxicity or pains on injection are typical drawbacks associated with cosolvents (9).
- Systems contain a large amount of surfactants to solubilize drugs by an increased wetting of the hydrophobic compound. But surfactants can also cause side effects. A typical example is the hypersensitivity reaction caused by the Cremophor EL® in Taxol® (10). Another example of surfactants is mixed micelles, for example, Valium® MM (11).
- In case of lipophilic drugs, a low melting point emulsification system such as microemulsions, self-emulsifying DDSs, or self-micro emulsifying DDSs, could be used. The drawbacks of these systems include batch-to-batch variability, chemical instabilities, and high surfactant concentration.
- Another approach is the use of liposomes to incorporate hydrophobic drugs in phospho-lipid bilayers of uni- or multilamellar vesicles. One example is the drug Amphotericin B, which is marketed as liposomal formulation Ambisome®.
- A specific approach is the formation of inclusion complexes, for example, with cyclodextrins. Cyclodextrines are cyclic oligomers of dextrose or dextrose derivatives, which can form a reversible, noncovalent association with poorly soluble drugs to solubilize them. Especially, the more water soluble & less toxic derivatives, such as sulfobutylether-β-cyclodextrin (Captisol™, CyDex, Inc.) and hydroxy-propyl-β-cyclodextrin (HP-β-cyclodextrin), are used in different pharmaceutical formulations (12). Sporanox® by Johnson and Johnson/Janssen (Itraconazole/HP-β-cyclodextrine) and Zeldox® by Pfizer (Ziprasidone/SBE-β-cyclodextrine; Captisol®) are examples of marketed products. In order to build this complex, it is in general required that the drug molecule fits into the cyclodextrin cavity. For that reason, this promising specific approach can be used only for a limited number of drugs. Another drawback of this technology is the high excipient level of the resulting product.

To sum up, the prementioned technologies are successfully applied for a number of drugs. The marketed products prove their acceptance and applicability. The success of a technology can be rated in two areas

1. Time between developing the technology and first market products and
2. Number of products on the market in total (or more precisely, number of products launched per year).

Based on these success criteria, the performance of most specific formulation approaches appears rather poor. Liposomes—rediscovered by Bingham in 1968— needed about 20 years to come to market. The number of products is relatively low, definitely distinctly behind expectations. Similar is the situation for cyclodextrines, currently moving forward with the new, better tolerated derivatives. These technologies are commonly used as primary strategies. However, they are more or less specific approaches for the solubilization of a certain drug candidate. They can be used only in compliance with certain requirements determined by the drug and route of administration; there is no universal formulation approach. Much smarter are nonspecific formulation approaches applicable to almost any drug molecule (apart from a few exceptions). Particle size reduction has been a nonspecific formulation approach for many years. The micronization of drugs is applied to increase their surface area. Increasing the surface area will proportionally increase the rate of dissolution and the rate of diffusion (absorption). Micronization means transfer of relatively coarse drug powder to micrometer crystals using colloid mills or jet mills. The mean diameter of such micronized drug powders is in the range of approximately 2 to 5 µm, corresponding to a size distribution of approximately 0.1 to 20 µm (13). Owing to their particle size distribution, such formulations in general cannot be used for intravenous (IV) injections. Micronization cannot improve the saturation solubility of a drug substance. In cases of practically insoluble pharmaceutical compounds or compounds of very low solubility, the effect of micronization on the bioavailability is not sufficient. For that reason, the next consequent step was to go down one further dimension in size, which means to reduce the particle size in the nanometer range. Since the 1980s, when drug nanoparticles were produced by List and Sucker (14) via precipitation, various techniques for the production of drug nanocrystals have been developed.

Advantages of nanocrystals

• Increased rate of absorption,
• Increased oral bioavailability,
• Rapid effect,
• Improved dose proportionality,
• Reduction in required dose,
• Reduction in fed/fasted variability,
• Rapid, simple and cheap formulation development
• Possibility of high amounts (30-40 %) of drug loading,

• Increased reliability. Usually side effects are proportional to drug concentration, so decreasing the concentration of active drug substances leads to an increased reliability for patients (15, 16).

• Applicability to all poorly soluble drugs because all these drugs could be directly disintegrated into nanometer-sized particles.

• Sustained crystal structure. Nanocrystal technology leads to an increase in dissolution rate depending on the increase in surface area obtained by reduction of the particle size of the active drug substance down to the nano size range preserving the crystal morphology of the drug (17).

• Improved stability. They are stable systems because of the use of a stabilizer that prevents reaggregation of active drug substances during preparation (18). Suspension of drug nanocrystals in liquid can be stabilized by adding surface active substances or polymers.

• Applicability to all routes of administration in any dosage form. Contrary to micronized drugs, nanocrystals can be administered via several routes. Oral administration is possible in the form of tablets, capsules, sachets or powder; preferably in the form of a tablet. Nanosuspensions can also be administered via the intravenous route due to very small particle size, and in this way, bioavailability can reach 100 %.

PHYSICOCHEMICAL PROPERTIES OF DRUG NANOCRYSTALS

The increased saturation solubility and the accelerated dissolution velocity are the most important differentiating features of drug nanocrystals. In general, the saturation solubility (c_s) is defined as a drug-specific constant depending only on the solvent and the temperature. This definition is only valid for drug particles with a minimum particle size in the micrometer range. A particle size reduction down to the nanometer range can increase the drug solubility.

The saturation solubility of solid particles depends on their particle radius and their lattice structure according to the Ostwald-Freunlich equation and the Kelvin equation

$$\ln\left(\frac{S}{S_0}\right) = \frac{2\upsilon\gamma}{rRT} = \frac{2M\gamma}{\rho rRT} \qquad (1)$$

Where S is the drug solubility at temperature T,

S_0 the solubility if $r = \infty$,

M the molecular weight of the compound,

υ the molar volume,

γ the interfacial surface tension, and

ρ the density of the compound

From the Ostwald-Freundlich equation, it can be concluded that a drug shows higher solubility if the particle radius is decreased. This effect is not substantial for larger particles but will be more pronounced for particles below 1 to 2 µm, especially well under 200 nm. Another important factor influencing the solubility is the crystalline structure of the drug. The higher the solid density and the melting point are, the lower the solubility. In contrast, a polymorph form with a lower packaging shows a higher molar volume and lower solid density (19).

The Kelvin equation can also be used to describe the correlation of increased saturation solubility by decreased particle size. The Kelvin equation describes the vapor pressure as a function of the curvature of liquid droplets in a gas phase. The vapor pressure increases with increasing curvature (decreasing particle size).

This can be transferred to solid drug particles in a liquid medium: the dissolution pressure increases with decreasing particle size (20).

$$\frac{dc_x}{dt} = \frac{DA}{h}(c_s - c_x) \qquad (2)$$

Where dc_x/dt is the dissolution velocity,

D the diffusion coefficient,

A the surface of the drug particle,

h the thickness of diffusional layer,

c_s the saturation solubility of the drug, and

c_x the concentration in surrounding liquid at time x.

The increased dissolution velocity is the characteristic feature of drug nano-crystals. The Noyes-Whitney equation [Equation (2)] describes now an increase in dissolution velocity is proportional to an increase in surface area. For example, when moving from a spherical 50 µm particle to micronized 5 µm particles, the total surface area enlarges by a factor of 10, moving to 500 nm nanocrystals by a factor of 100. The decrease in the diffusional distance h is an additional factor accelerating the dissolution velocity. According to the Prandtl equation [Equation (3)], the diffusional distance h is reduced with increasing curvature of ultrafine

particles. Together with the increased saturation solubility of ultrafine particles, the concentration gradient in the Noyes-Whitney equation is significantly increased. For that reason, nanonization can distinctly increase the dissolution velocity of poorly soluble drugs:

$$h_H = k\left(\frac{L^{1/2}}{V^{1/3}}\right) \quad (3)$$

Where h_H is the hydrodynamic boundary layer thickness

V the relative velocity of the flowing liquid against a flat surface

k a constant

L the length of the surface in the direction of flow

Drug nanocrystals can be used for a chemical stabilization of chemically labile drugs. The drug paclitaxel can be preserved from degradation when it is formulated as a nanosuspension (21,22). The same result was found for the chemically labile drug omeprazole. When formulated as a nanosuspension, the stability was distinctly increased in comparison to the aqueous solution (23). The increased stability can be explained by a shield effect of the surfactants and the drug protection by a mono-layer made of degraded drug molecules which reduce the accessibility for destructive agents (24)

FORMULATIONS FOR DRUG NANOCRYSTALS

In the formulation of drug nanocrystals polymers play an important role. Polymers used in formulation of drug nanocrystals may be classified as either

(i) natural and synthetic, or

(ii) Biodegradable and non biodegradable.

Examples of naturally occurring biodegradable and biocompatible polymers used to prepare nanocrystals include: cellulose, gelatin, pullulan, chitosan, alginate, and gliadin. The characteristics and performance, particularly in vivo, of nanoparticles prepared using natural polymers may be less predictable as these polymers may vary widely in chemical composition and hence, physical properties. In addition, natural polymers are often mildly immunogenic. As a result, synthetic polymers are also more easily designed for specific applications, such as controlled rates of dissolution, permeability, degradation, and erosion, as well as for targeting.

Examples of synthetic biodegradable polymers used to prepare nanocrystals include:

polylactide (PLA), poly-(lactide-co-glycolide) (PLGA), polyanhydrides, poly-ε-caprolactone, and polyphosphazene.

Surfactants or stabilizers have to be added for the physical stability of the produced nanosuspensions. In the production process the coarse drug powder is dispersed by high-speed stirring in a surfactant/stabilizer solution to yield a macrosuspension. The choice of surfactants and stabilizers depends not only on the properties of the particles to be suspended (e.g., affinity of surfactant/stabilizer to the crystal surface) but also on the physical principles (electrostatic vs. steric stabilization) and the route of administration. . Polysorbate 80 (nonionic), sodium laurylsulfate (SLS) and docusate sodium (DOSS) (both anionic) are some examples of suitable surfactant stabilizers for physical stability. Also, surfactants of-ten help in the wetting, electrostatic stabilization and dispersion of the drug particles, which are usually very hydrophobic. Hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC), povidone (PVP K30), and pluronics (F68 and F127) is polymers suitable for use as stabilizers. HPMC E3, Povidone, DOSS, and SLS are some of the stabilizers that have been used in the nanocrystal formulations of drugs that are on the market today (25).

In order to show their advantages in vivo, the drug nanocrystals need to be transferred into the right dosage form. Nanosuspensions can be directly used as oral suspensions to overcome the difficulties of swallowing tablets by pediatric or geriatric patients. One example is Megace® (Bristol Meyers Squibb), an oral suspension of megestrol acetate, used for the treatment of HIV-associated anorexia and cachexia. The application of these nanosuspensions can improve the solubility of the drug and the dissolution rate; additionally, suspensions can be applied for reasons of taste-masking. Nanosuspensions can also be used directly for parenteral drug administration. Although nanosuspensions have shown a sufficient long-term stability without Ostwald ripening, for intravenous products a lyophilization step is recommended in order to avoid aggregation or caking of settled drug nanocrystals. The lyophilized product can be easily reconstituted before use by adding isotonic water, aqueous glucose solution, or other reconstitution media (26, 27).

Without question, both the patients and the marketing experts prefer the oral administration of traditional dosage forms. Hence, to enter the pharmaceutical market successfully in most cases drug nanocrystals have to be formulated as traditional products, such as tablets or capsules. A perfect solid dosage form should preserve the in vivo performance of drug nanocrystals. When reaching the target part of the GI tract, the dosage form should release the drug nanocrystals as a fine, nonaggregated suspension. Otherwise, self agglomeration or aggregation can impair the drug release (28). Using nanosuspensions as granulation fluid for a further tablet production is a very simple approach. The

nanosuspension is admixed to binders and other excipients, and the granules are finely compressed to tablets. This dosage form is limited in the maximum achievable drug content. A maximum drug content of about 50% or less is suggested in order to ensure a complete disintegration into a finely dispersed suspension (29). Nanosuspensions can also be used for the production of matrix pellets (Fig. 1) or as layering dispersions in a fluidized bed process. After the pellet preparation, the cores can be coated with several polymers in order to modify the release profile of the final formulation (30-32).

A very smart formulation approach is the Nanopure® technology. Nanocrystals produced in nonaqueous media, such as liquid PEG or oils (e.g., Miglyol), can be directly filled into gelatin or HPMC capsules. The production of drug nanocrystals in melted PEGs is a new strategy for the production of final dosage forms containing drug nanocrystals. After performing the high-pressure homogenization in melted PEG at about 60°C, the mixture can be solidified. The resulting matrix, fixing the drug nanocrystals in separated state, can be compressed to tablets or directly filled into capsules (33). Spray-drying of the nanosuspensions is another cost-effective approach to transfer nanosuspensions into dry products. The drug nanocrystals can directly be produced by high-pressure homogenization in aqueous solutions of water-soluble matrix materials, for example, polymers [polyvinylpyrrolidone, polyvinylalcohol or long-chained PEG, sugars (saccharose, lactose) or sugar alcohols (mannitol, sorbitol)]. Afterwards, the resulting nanosuspension can be spray-dried under appropriate conditions. The dry powder, composed of drug nanocrystals embedded in a water soluble matrix, can be filled in hard gelatin capsules for oral administration (34).

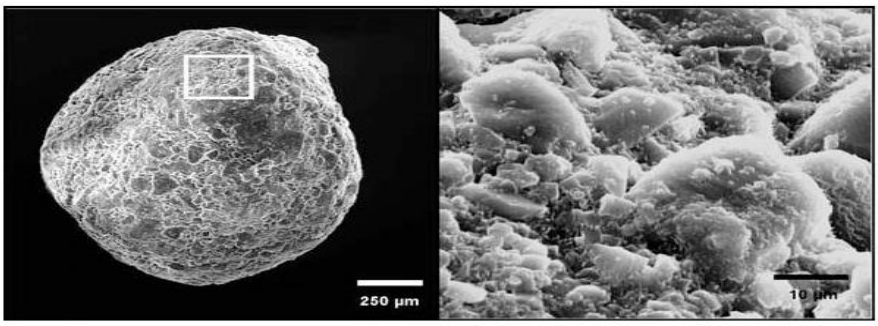

FIGURE 1 SEM photograph of uncoated matrix core containing drug nanocrystals (Left) overview (magnification 60×),

(Right) detailed magnification (1000×) showing drug nanocrystals combined with the binder material.

Another attractive approach using the spray-drying principle is described as “direct compress technology” (35). Lactose and other matrix-forming materials, such as micronized polymer powders or lipids, are admixed to the prior-produced nanosuspension. The resulting suspension is transferred into a drug-matrix-compound by spray-drying. Subsequently, the free-flow able powder can be used for direct compression of fast dissolving or prolonged release tablets. Alternatively, the powder can also be filled into hard gelatin capsules.

NANOCRYSTALS PREPRATION METHODS

Several preparation methods for drug nanocrystals have been investigated. These are schematically depicted in Figure 2.

Today, implemented preparation methods of nanocrystal formulations can be classified as “bottom up”, “top-down”, “top down and bottom up” and “spray drying”. “Bottom up” technology begins with the molecule; active drug substance is dissolved by adding an organic solvent, and then, solvent is removed by precipitation. “Top-down” technology applies dispersing methods by using different types of milling and homogenization techniques. “Top-down” technology is more popular than “Bottom up” technology; it is known as “nanosizing”. In other words, it is a process which breaks down large crystalline particles into small pieces. In “top down and bottom up” technology, both methods are utilized together (25). Spray drying is also a method for preparing drug nanocrystals, which is faster and more practical compared to the other methods.

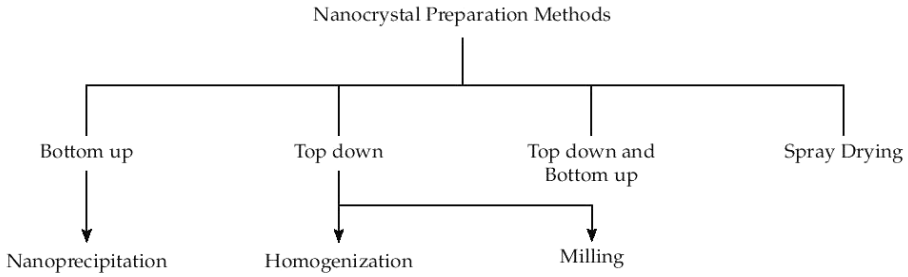

Figure 2 Preparation methods of drug nanocrystals

Bottom up Technology

“Bottom up” technology relies on precipitation (36, 37).

The classical precipitation process, known as “via humida paratum,” is actually a very old pharmaceutical procedure. Later, this basic idea was applied for the production of nanocrystalline drug particles (38). The first application of the precipitation technique was

developed by List and Sucker (39). It is known as hydrosol technology, and the IP is owned by Sandoz (now Novartis). A poor water-soluble drug is dissolved in an organic medium, which is water-miscible. A pouring of this solution into a nonsolvent, such as water, will cause a precipitation of finely dispersed drug nanocrystals. As simple as the particle formation process is, the preservation of the nanocrystalline particle size is difficult. The fine particles tend to grow up, driven by a phenomenon called "Ostwald ripening." This is a process where small particles are dissolved in favor of larger particles. Sucker (40) suggested immediate lyophilization to preserve the particle size.

The crystalline state of the particles obtained by the precipitation process can be controlled. Depending on the employed method, amorphous drug nanoparticles can also be generated (41). Beta-carotene is dissolved in a water-miscible organic solvent together with digestible oil. This solution is admixed to an aqueous solution of a protective colloid (gelatin) causing a precipitation of amorphous nanoparticulate beta-carotene. After an annealing step and spray-drying, a stable amorphous product can be obtained. This NanoMorph® technology, invented by Auweter et al., is used by the company Soliqs.

Another approach to preserve the size of the precipitated nanocrystals is the use of polymeric growth inhibitors, which are preferably soluble in the aqueous phase. The increased viscosity of the aqueous phase can reduce particle growing. The resulting suspension is subsequently spray-dried to obtain a dry powder with a relatively high drug loading (42). Using this technique, a tremendous increase in dissolution rate (from 4% to 93% within 20 minutes) was shown for a poor water-soluble drug ECU-01 (43). Although the feasibility of preparing drug nanocrystals by precipitation has been shown by many groups, no commercial drug product using this technology has entered the market. To use the prementioned methods, it is required that the drug is soluble in at least one water-miscible solvent. This is often not the case for NCEs. Many drugs are simultaneously poorly soluble in aqueous and nonaqueous media. Even if there is a suitable solvent available, it is difficult to remove this solvent completely. Solvent residues can be potential risk factors for drug alteration and toxic side effects. In addition, in cases of amorphous drug nanoparticles, it is seen as very critical to preserve the amorphous character throughout the shelf life of a product. Recrystallization would impair the oral bioavailability. This effect is less critical in food products because of less strict regulatory requirements that allow more tolerance.

Top down Technology

As it is shown in the Figure 2, "top down" technology can be applied by either homogenization or milling.

Homogenization

High-pressure homogenization is a technology that has been applied for many years in various areas for the production of emulsions and suspensions. A distinct advantage of this technology is its ease for scaling up, even to very large volumes. High-pressure homogenization is currently used in the food industry, e.g., homogenization of milk. In the pharmaceutical industry parenteral emulsions are produced by this technology. Commercial products such as Intralipid and Lipofundin possess a mean droplet diameter in the range of 200-400 nm (photon correlation spectroscopy data) (44). In the mid-1990s of the last century drug nanosuspensions produced with high-pressure homogenization were developed (45-49). Typical pressures for the production of drug nanosuspensions are 1000-1500 bar (corresponding to 100-150 Mpa, 14504-21756 psi); the number of required homogenization cycles varies from 10 to 20 depending on the properties of the drug. Most of the homogenizers used are based on the piston-gap principle; an alternative is the jet-stream technology (Fig. 3).

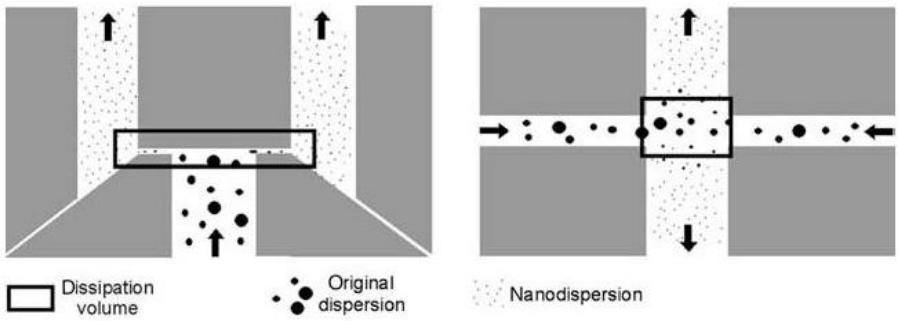

Figure 3 Basic homogenization principles: piston-gap (left) and jet-stream arrangement (right). In the piston-gap homogenizer the macrosuspension coming from the sample container is forced to pass through a tiny gap (e.g., 10 mm); particle diminution is affected by shear force, cavitation, and impaction. In jet-stream homogenizers the collision of two high-velocity streams leads to particle diminution mainly by impact forces.

The Microfluidizer (Microfluidics^{TM} Inc., U.S.A.) is based on the jet-stream principle. Two streams of liquid collide, diminution of droplets or crystals is achieved mainly by particle

collision, but occurrence of cavitation is also considered. The Micro fluidizer has also been described for the production of drug nanosuspensions; however, according to the patent 10-50 cycle passes were required (50). Such a high cycle number is not convenient for the production scale. The Micro fluidizer can be used for the production of drug nanosuspensions in the case of soft drugs. In the case of harder drugs, a larger fraction of particles in the micrometer range remain, which do not exhibit the increase in saturation solubility because of their too large size.

For many years cavitation was considered as the major force leading to particle diminution in the high-pressure homogenization process. Consequently, most high pressure homogenization patents in various application areas focus on water as a dispersion medium.

In the piston-gap homogenizer the liquid is forced through a tiny homogenization gap, typically in the size range of 5-20 mm (depending on the pressure applied and the viscosity of the dispersion medium). Using a Micron Lab 40 the suspension is supplied from a metal cylinder by a piston, the cylinder diameter is approximately 3 cm. The suspension is moved by the piston having an applied pressure between 100 and 1500 bar. In principle the piston-gap homogenizer corresponds to a tube system in which the tube diameter narrows from 3 to 5-20 mm. Accor-ding to the Bernoulli equation, the streaming velocity and dynamic pressure increase extremely, the static pressure in the gap falls below the vapor pressure of water at room temperature. A liquid boils when its vapor pressure is equal to the static pressure, which means water starts boiling in the gap at room temperature leading to the formation of gas bubbles. The formation of gas bubbles leads to pressure waves disrupting oil droplets or disintegrating crystals. When leaving the homogenization gap, the static pressure increases to normal air pressure, which means the water does not boil anymore and the gas bubbles collapse. Collapsing of the gas bubbles (implosion) leads again to shock waves contributing to diminution. There are different definitions of cavitation in the literature, describing cavitation either as the formation of gas bubbles in high streaming liquids or as the formation and subsequent implosion of these gas bubbles.

At the end of the 1990s it was found that similar efficient particle diminution can be achieved by homogenization in nonaqueous media such as oils and liquid polyethylene glycols (PEGs), which means media with low vapor pressure. In the case of low vapor pressure liquids, the cavitation in the homogenization gap is distinctly reduced or does not exist at all. Figure 4 shows the change in static pressure when homogenizing in water as dispersion medium (left)

and in a low-vapor liquid, whereas the static pressure does not fall below the vapor pressure (right).

Based on the aforementioned, cavitation does not seem to be essential for a diminution effect. Major forces are droplet or particle collision and the shear forces occurring in this highly turbulent fluid in the gap possessing a high kinetic energy. Homogenization in nonaqueous liquids has advantages for certain pharmaceutical final dosage forms. Preparation of drug nanocrystals in PEGs or oils (e.g., Miglyol 812 or 829) leads to nanosuspensions that can directly be filled into capsules (see the following) (51, 52). It is also possible to homogenize in melted nonaqueous matrices, which are solid at room temperature. Solidification of such a matrix leads to a fixation of drug nanocrystals in the solid matrix, thus minimizing or avoiding crystal contact and subsequent crystal fusion/growth

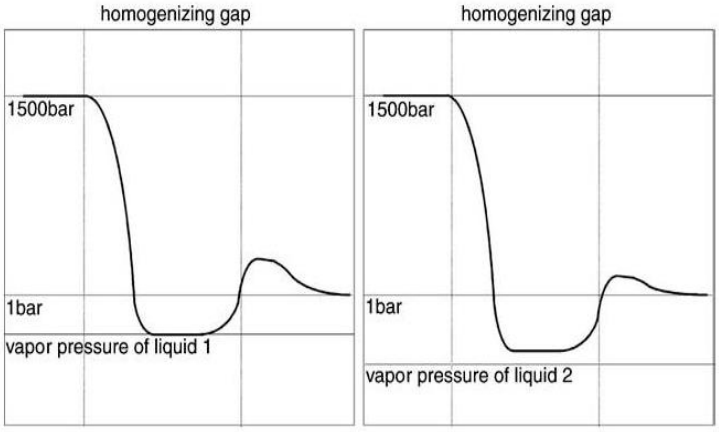

Figure 4 Variation of the static pressure (—) within the homogenizing gap. In the case of water the static pressure falls below the vapor pressure (left), whereas in the case of low-vapor media (right) the static pressure stays above the vapor pressure.

As a consequent next step, after homogenization in water (100% water) and homogenization in nonaqueous media (0% water), homogenization was performed in mixtures containing different percentages of water (1-99% water). The dispersion media were water mixed with water-miscible liquids (e.g., alcohols, glycerol). Preparation of drug nanosuspensions in water-ethanol mixtures is favorable for producing dry products, because later the spray drying can be performed under milder conditions when using such a mixture. Homogenization in water-glycerol mixtures (2.25% of water-free glycerol) leads to isotonic drug nanosuspensions for parenteral administration.

Pearl/Ball-milling technology for the production of drug nanocrystals

Traditional equipment used for micronization of drug powders such as rotor-stator colloid mills (Netzsch) or jet mills (Retsch) are of limited use for the production of nanocrystals. For example, jet milling leads to a drug powder with a size range of roughly between 0.1 and 20mm, containing only a very small fraction of about 10% in the nanometer range (53). However, it could be shown when running a pearl mill over a sufficiently long milling time that drug nanosuspensions can be obtained (54, 55). These mills consist of a milling container filled with fine milling pearls or larger-sized balls. The container can be static and the milling material is moved by a stirrer; alternatively, the complete container is moved in a complex movement leading consequently to movement of the milling pearls.

There are different milling materials available, traditionally steel, glass, and zircon oxide are used. New materials are special polymers, i.e., hard polystyrene. A problem associated with the pearl milling technology is the erosion from the milling material during the milling process. Buchmann et al. (56) reported about the formation of glass microparticles when using glass as milling material. Normally, product containers are made of steel and can be covered with various materials to fulfill the required quality specifications of the formulation. Surfactants or stabilizers have to be added for the physical stability of the produced nanosuspensions. In the production process the coarse drug powder is dispersed by high-speed stirring in a surfactant/stabilizer solution to yield a macrosuspension. The choice of surfactants and stabilizers depends not only on the properties of the particles to be suspended (e.g., affinity of surfactant/stabilizer to the crystal surface) but also on the physical principles (electrostatic vs. steric stabilization) and the route of administration. In general, steric stabilization is recommended as the first choice because it is less susceptible to electrolytes in the gut or blood. An electrolyte reduces the zeta potential and subsequently impairs the physical stability, especially of ionic surfactants. In many cases an optimal approach is the combination of a steric stabilizer with an ionic surfactant, i.e., the combination of steric and electrostatic stabilization. There is a wide choice of various charged surfactants in case of drug nanocrystals for oral administration. Even relatively ‘‘nasty’’ surfactants, such as the membrane damaging SDS, can be used, of course within the concentration accepted for oral administration, e.g., the formulation of Emend (57). SDS as a low molecular weight surfactant diffuses fast to particle surfaces; it has excellent dispersion properties. Adsorption onto the particle surface leads to high zeta potential values providing good physical stabilities. In case of parenteral drug nanocrystals, the choice is limited; e.g., for

intravenous injection, accepted are lecithins, Poloxamer 188, Tween[1] 80, low molecular weight polyvinylpyrrolidone (PVP), sodium glycocholate (in combination with lecithin). Drug nanocrystal suspensions for parenteral administrations need to be sterile, depending on the administration route and the volume they need to be pyrogene free. Production of parenteral drug nanosuspension using pearl mills is much more tedious compared to producing oral drug nanosuspensions. The equipment needs to be sterilized and the product needs to be separated from the milling pearls by a preferentially aseptic separation process. A terminal sterilization by autoclaving is only possible with a number of products (58). The use of an ionic stabilizer such as lecithin is recommended when autoclaving nanosuspensions. The autoclaving temperature of 121°C leads to dehydration of steric stabilizers, which reduces their ability to stabilize the suspensions. Gamma irradiation of nanosuspensions is an alternative, but is less favoured by the pharmaceutical industry due to regulatory requirements (e.g., proof of absence of toxic radicals, etc.). From the industrial point of view, in many cases a well-documented aseptic production is easier for the production of formulations for parenteral administration than gamma irradiation.

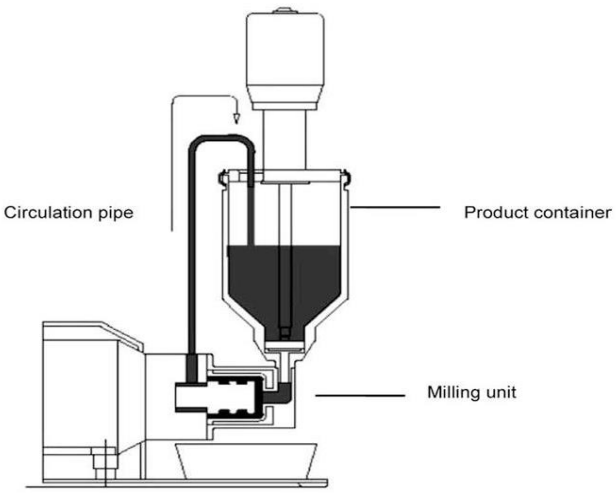

Figure 5 DISPERMAT SL: schematic view of a bead mill using recirculation method.

There are a number of pearl mills available on the market, ranging from laboratory-scale to industrial-scale volumes. The ability for large-scale production is an essential prerequisite for the introduction of a product to the market. One advantage of the pearl mills, apart from being low-cost products, is their ability for scaling up. Assuming, for reasons of simplicity, hexagonal packaging of the milling pearls, 76% of the milling chamber volume will be filled by the pearls. In case of a 1000 L mill this corresponds to 760 L milling material; based on the apparent density of zircon oxide pearls being 3.69 kg/L, this

corresponds to 2.8 tons of milling material. Figure 1 shows the solution for this problem, a pearl mill with an external suspension container. The suspension is continuously pumped through the pearl mill. This approach reduces the weight of the pearl mill itself, but it prolongs the milling times.

Disadvantages of this method are
Potential contamination of the product by erosion of the milling material,
Relatively long milling times for hard crystalline drugs, and
Limited scaling up due to the weight of large scale pearl mills

Top Down and Bottom Up Technology

In "top down and bottom up" technology, both methods are used together. NanoEdge® is a product obtained by such a combination technology. As can be inferred, precipitation is followed by high pressure homogenization in this technology (59).

Nanoedge® Technology

Baxter's NANOEDGE® process relies on the combination of a micro precipitation technique with a subsequent annealing step by applying high shear and/or thermal energy (60). A fine suspension is formed by adding an organic solution of the water-insoluble drug to an antisolvent, for example, aqueous surfactant solution. Depending on the precipitation conditions, either small amorphous or crystalline drug particles in the nanometer range or friable needle-like crystals in the micrometer range are formed. Consequently, the following high-energy input can have two effects on the preformed particles. Small amorphous or crystalline drug particles will be preserved in size by an annealing step without changing the mean diameter. It could be shown that the tendency to crystal growth can be reduced by energy input after the precipitation step. In case long friable needle-like crystals are obtained, they will be reduced in size by the high-energy input using high-pressure homogenizers. According to the patent (60), particle sizes in the range of 400 to 2000 nm can be obtained. The organic solvent utilized has to be carefully removed from the final nanosuspension without changing the particle size of the drug nanocrystals. Otherwise, crystal growth can be promoted by an increased solubility of the drug. Any content of the organic solvent dissolved in the aqueous phase can act as a "cosolvent," leading to an increased tendency to Ostwald ripening. Also, toxic effects can be caused by potential solvent residues, especially if the nanosuspension is the final product. For these reasons, the NANOEDGE® process is

particularly suitable for drugs that are soluble in nonaqueous media possessing low toxicity, such as N-methyl-2-pyrrolidinone.

Spray- Drying

For the production of tablets, an aqueous nanosuspension can be used as granulation fluid or a dry form of the nanosuspension, powder, or granulate can be employed. Starting from an aqueous macrosuspension containing the original coarse drug powder, surfactant, and water-soluble excipient, the homogenization process can be performed in an easy one step yielding a fine aqueous nanosuspension. In a subsequent step the water has to be removed from the suspension to obtain a dry powder. One method of removing the water from the formulation is freeze drying, but it is complex and cost-intensive leading to a highly sensitive product (61, 62). Another simple and most suitable method for the industrial production is spray drying. The drug nanosuspension can directly be produced by high-pressure homogenization in aqueous solutions of water-soluble matrix materials, e.g., polymers [PVP, poly-vinylalcohol or long chained PEG, sugars (saccharose, lactose), or sugar alcohols (mannitol, sorbitol)]. Afterward the aqueous drug nanosuspension can be spray dried under adequate conditions; the resulting dry powder is composed of drug nanocrystals embedded in a water-soluble matrix (63). Figure 6 schematically represents the whole production process of drug nanocrystal-loaded spray-dried compounds.

The loading capacity of the solid powder with drug nanocrystals can be adjusted by varying the concentrations of excipient and surfactant in the original aqueous nanosuspension. One aim of a solid nanoparticulate system is releasing the drug nanocrystals after administration in the gastrointestinal tract (GI) as a fine non aggregated suspension; the other is to increase the physical stability for long-term storage. Contact of the drug nanocrystals is averted by fixation within the matrix. Thereby, the probability of physical instabilities as, e.g., aggregation and ripening are in principle clearly avoided or minimized to a negligible extent. However, appropriate investigations have shown a relation between the loading capacity of the compounds and the releasing behavior, as well as the storage stability. Exceeding a certain maximum loading capacity of the matrix with drug nanocrystals has an increasing negative effect on particle crystal growth and on release as fine dispersion (64).

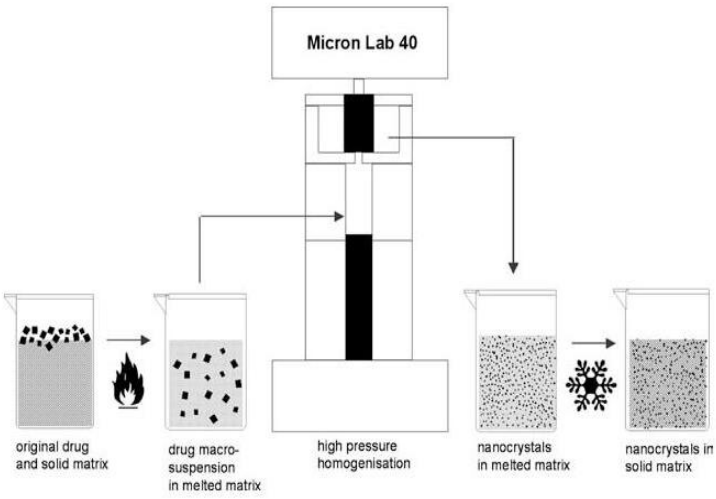

Figure 6 Two-step process of the production of drug nanocrystalloaded compounds: the drug nanosuspension obtained by high pressure homogenization (Micron Lab 40) is further processed by spray drying using a Mini Buchi. Drug nanocrystals embedded in the matrix are obtained.

APPLICATIONS OF DRUG NANOCRYSTALS

Application for Oral Delivery

The oral route is the most important and preferred route of administration. The formulation of drug nanocrystals can impressively improve the bioavailability of per orally administered poorly soluble drugs. In 1995, Liversidge and Cundy (65) reported an increase in bioavailability for the drug Danazol from 5.1 ± 1.9% for the conventional suspension to 82.3 ± 10.1% for the nanosuspension. The increased dissolution velocity and saturation solubility lead to fast and complete drug dissolution, an important prerequisite for drug absorption.

Whenever a rapid onset of a poorly soluble drug is desired, the formulation of drug nanocrystals can be beneficial, for example, in case of analgesics. The analgesic naproxen, formulated as a nanosuspension, has shown a reduced t_{max} but simultaneously approximately threefold increased AUC in comparison to a normal suspension (Naprosyn®) (66).

Besides the faster onset of action, the naproxen nanosuspension has also shown a reduced gastric irritancy (67, 68). If absorption windows limit the drug absorption or by food effects, drug nanocrystals have advantages in comparison to conventional suspensions. Wu et al. have reported reduced fed-fasted ratio and an improved bioavailability for nanocrystalline aprepitant (MK-0869), the active ingredient in Emend®, in beagle dogs. Another important advantage of drug nanocrystals is their adhesiveness and the increased residence time, which

can positively influence the bioavailability. The mucoadhesiveness can be raised by the use of mucoadhesive polymers in the dispersion medium (69, 70). Additionally the utilized mucoadhesive polymers can prevent the drug from degradation. The reduced particle size can be also exploited for improved drug targeting, as reported for inflammatory tissues (71) or the lymphatic drug uptake (72).

Parenteral Administration of Drug Nanocrystals

The parenteral application of poorly soluble drugs, particularly intravenous (IV) administration of practically insoluble compounds, using cosolvents, surfactants, liposomes, or cylcodextrines, is often associated with large injection volumes or toxic side effects. Carrier-free nanosuspensions enable potential higher loading capacity compared to other parenteral application systems. Using nanosuspensions, the application volume can be distinctly reduced compared to solutions (23). To fulfill the distinctly higher regulatory hurdles, the drug nanocrystals need to be produced in an aseptic process. Alternatively, nanosuspensions can be sterilized by autoclaving (73) or alternatively by gamma irradiation as well as sterile filtration (74). When a drug is administered as a nanosuspension, the rapid dissolution of the nanocrystals will mimic the plasma concentration profile of a solution. Drug nanosuspensions can be formulated with accepted surfactants and polymeric stabilizers for IV injection. In contrast, solutions of poorly soluble drugs require the use of cosolvents and/or high surfactant contents (e.g., Chremophor EL in Taxol®), which can cause undesired side effects (75). Comparing the toxicity of Taxol® with a paclitaxel nanosuspension, the latter has shown a distinctly reduced toxicity. The nanosuspension was much better tolerated, resulting in an approximately doubled LD50 value (26). The same effect of increased tolerated dose was found for the antifungal drug itraconazole. Itraconazole is marketed as Sporanox IV® by Janssen Pharmaceutica Products, L.P., an inclusion complex of itraconazole and 2-hydro-xypropyl-β-cyclodextrine (HP-β-CD). The product exhibits a significant acute toxicity above 10 mg/kg and an LD50 value lower than 40 mg/kg when administered as a bolus in the caudal vein of rats. In contrast, a 1% nanosuspension of itraconazole could be administered up to 320 mg/kg without animal mortality. Besides the decreased acute toxicity, the nanosuspension has also shown a prolonged effect, whereby the administration intervals could be extended almost three times in com-parison to the daily administration of Sporanox IV® (19). Comparing a clofazimine nanosuspension with a liposomal formulation, both are similarly effective in the treatment of artificially induced Mycobacterium avium infections. The targeting to the reticuloendothelial system, the lung, liver, and spleen was comparable to

the liposomal formulation (76). Furthermore, a special targeting can be achieved by a surface modification using the concept of "differential protein adsorption." A surface modification of drug nanocrystals with the surfactant Tween 80 leads to a preferential adsorption of apolipoprotein E. This protein adsorption enables a targeted delivery of drug nanocrystals to the brain. Atovaquone drug nanocrystals modified with Tween 80 have shown an excellent efficacy in the treatment of Toxoplasmosis (77).

Drug Nanocrystals for Pulmonary Drug Delivery

Delivery of water-insoluble drugs to the respiratory tract is very important for the local or systemic treatment of diseases. Many important drugs for pulmonary delivery show poor solubility simultaneously in water and nonaqueous media, for example, important corticosteroids such as budesonide or beclomethasone dipropionate. In the past, most of these drugs were administered as aerosols, but in compliance with the Montreal Protocol of 1987 the use of chlorofluorocarbon (CFC) must be avoided. Therefore, alternatives such as dry powder inhalers or metered dose inhalers without CFC (MDI) were developed. These systems are filled with micronized drug powders produced by jet-milling. The mean particle size in the lower micrometer range (3-25 µm) results in a significant oropharyngeal deposition of larger particles leading to increased occurrence of candidasis. Additionally, the oral deposition of the drug leads to gastrointestinal (GI) drug absorption followed by systemic side effects (78). Nanosuspensions can be successfully applied to overcome these problems. The nebulization of nanosuspensions generates aerosol droplets of the preferred size loaded with a large amount of drug nanocrystals. A Usingnebulized nanosuspension, the respirable fraction is distinctly increased in comparison to conventional MDIs (78). The smaller the particles size of the drug nanocrystals, higher the drug loading of the aerosol droplets (79, 80). Therefore, the required nebulization time is distinctly reduced (81). Besides this, drug nanocrystals show an increased mucoadhesiveness, leading to a prolonged residence time at the mucosal surface of the lung.

Other Administration Routes

Dermal nanosuspensions are mainly of interest if conventional formulation approaches fail. The use of drug nanocrystals leads to an increased concentration gradient between the formulation and the skin. The increased saturation solubility leads to "supersaturated" formulations, enhancing the drug absorption through the skin. This effect can further be enhanced by the use of positively charged polymers as stabilizers for the drug nanocrystals.

The opposite charge leads to an increased affinity of the drug nanocrystals to the negatively charged stratum corneum (unpublished data).

The ocular delivery of nanoparticles, including drug nanocrystals, is also of high interest. The development of such colloidal delivery systems for ophthalmic use aims at droppable dosage forms with a high drug loading and a long lasting drug action. The adhesiveness of the small nanoparticles, which can be further increased by the use of mucoadhesive polymers, leads to a more consistent dosing. Blurred vision can be reduced by the use of submicron-sized drug particles (82, 83).

RECENT ADVANCEMENTS

Production In Nonaqueous Liquids

To avoid the removal of water after high-pressure homogenization in aqueous media, homogenization can be performed directly in nonaqueous media. A number of nonaqueous media are suitable as dispersion media for drug nanocrystals. For example, PEG and triglycerides or self-emulsifying drug delivery systems are ideal liquid candidates and are suited for direct filling of hard or soft gelatin capsules (84). The production process can be easily performed similar to the process in water.

Nanopure® XP Technology

Considering the commonly used particle size reduction techniques, the production of nanosuspensions is in most cases associated with a high-energy input (high-speed media mills, high-pressure homogenization) and a relatively long period between the drug synthesis and the final product. A micronized drug material (size 10-100 µm) is recommended for milling and high-pressure homogenization processes (85, 86). Therefore, the drug often has to be jet-milled before the nanonization. Impurities caused by an abrasion from the milling material or solvent residues from the precipitation process are undesirable. They can causes side effects especially if the drug is administered for the treatment of chronic diseases. The minimal achievable particle size is significantly determined by the hardness of the drug. In cases of very hard drugs, an increasing energy input (by extending the milling time or the number of homogenization cycles) will not lead to a smaller particle size. In general, the smallest achievable size of nanocrystals is around 200 nm; only under special conditions can about 100 nm be produced. However, especially crystals below 100 nm would show an extremely fast dissolution and simultaneously a great increase in saturation solubility (19). Therefore, such particles are of high commercial interest.

In 2005, Möschwitzer (87) developed a new combination method for the production of drug nanosuspensions, which is now owned by PharmaSol GmbH. The Nanopure® XP technology (process variant: H42) enables extension of the performance of the Nanopure® technology to very hard and crystalline materials. Modification of the starting material by an evaporation process before the subsequently performed high-pressure homogenization can significantly reduce the number of homogenization cycles (88). Owing to the reason that the solvent will be removed completely before homogenization by the evaporation process, various solvents can be used for the modification process without restrictions due to toxicity reasons. Additionally, excipients can be added to the drug solution to increase the number of crystal imperfections upon drying. Figure 7 makes clear the effectiveness of the new combination method in comparison to the classical high-pressure homogenization. The application of the H42 technology leads to distinctly reduced particle size by a simultaneous reduction of the required number on homogenization cycles. This will consequently reduce the production costs and the wear on the homogenization equipment.

H96 is another technology belonging to the Nanopure® XP platform. The technology, developed in 2005 by Möschwitzer and Lemke (89), is a modified process based on high-pressure homogenization. By using this undisclosed technology, it was shown that drug nanocrystals below 100 nm can be produced by high-pressure homogenization.

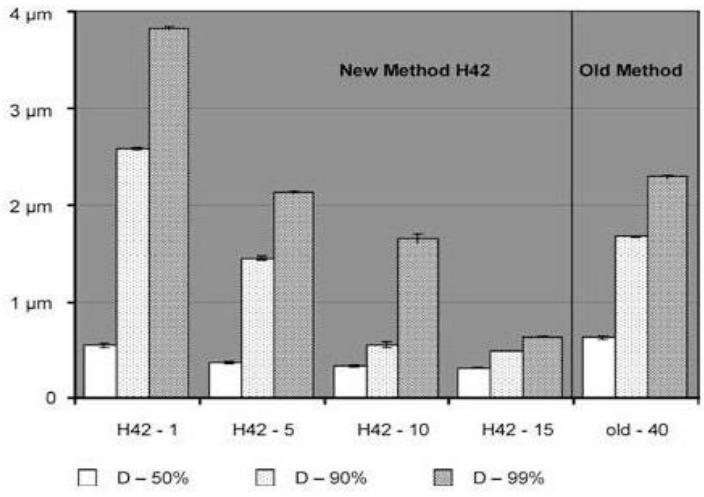

FIGURE 7 Comparison of the new homogenization technology H42 (left) with the conventional homogenization in water (right), performed in piston-gap homogenizer, influence of cycle numbers, results represent the laser diffractometry diameters (volume-weighted, Coulter Ls 230, Beckman-Coulter, Germany).

Source: From Ref. 90

FIGURE 8 Two nanosuspensions composed similarly, produced by high-pressure homogenization, conventional method (left) versus translucent nanosuspension (particle size well below 100 nm) resulting from H96 technology. The red laser beam is reflected by the tiny nanocrystals.

Source: From Ref. 90.

H96 results in a translucent nanosuspension in comparison to another nanosuspension produced using the Dissocubes® technology. Figure 8 shows two nanosuspensions. The left nanosuspension was produced applying the conventional high pressure homogenization in water; the right nanosuspension was produced by using the H96 technology. Although both formulations are being composed similarly, the right formulation is translucent due to the significantly reduced particle size. The particle size analysis also provides the evidence of the performance of the H96 method. Almost 99% of the particles were smaller than 100 nm [laser diffractometry (LD) D99%, volume size distribution, LS 230, Beckman-Coulter, Germany unpublished data with permission from Ref. 90]. A very small particle size and a narrow range of particle size distribution can be obtained by using the H96 technology.

Few Examples of Drug Nanocrystals formulations:

Product	**Drug**	**Indication**	**Technology by/ license to**	**Year of FDA Approval**
Rapamune®	Sirolimus	Immunosuppressant	Elan/Wyeth	2000
Emend®	Aprepitant	Antiemetic	Elan/Merck	2003
Tricor®	Fenofibrate	Treatment of high cholesterol and high triglyceride levels	Elan/Abbott	2004
Megace ES®	Megestrol Acetate	Palliative treatment of some breast and uterine cancers	Elan/Par Pharm	2005
Triglide®	Fenofibrate	Treatment of high cholesterol and high	SkyePharma/ First Horizon	2005

		triglyceride levels	Pharmaceuticals	
Invega Sustenna®	Paliperidone palmitate	Treatment of schizophrenia	Elan/ Johnson and Johnson	2009

CONCLUSION

According to literature, about 60 % of all synthesized drug candidates are poorly water-soluble. Poor aqueous solubility is clearly recognized by the pharmaceutical industry as a major problem. Thus, it appears that nanocrystals technology will continue to thrive as a useful tool in pharmaceutics for the improvement of drug solubility, oral absorption, and hence, bioavailability. Almost any drug can be reduced in size to the nanometer range. Many insoluble drug candidates are in clinical trials formulated as drug nanocrystals The fact that this technology has many advantages; such easy production and scale up, and low cost, make this approach a very attractive means for solving a very serious problem of drugs, poor water-solubility in conjunction with low oral absorption and bioavailability.

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