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European Journal of Pharmaceutics and Biopharmaceutics 62 (2006) 3–16
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Review article

Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation

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Received 23 May 2005; accepted in revised form 30 May 2005
Available online 29 August 2005

Abstract

For many new chemical entities (NCE) of very low solubility oral bioavailability enhancement by micronisation is not sufficient, the next step taken was nanonisation. The production of drug nanocrystals by bottom up techniques (precipitation) is briefly described, main focus is given on particle diminution by high pressure homogenisation. Homogenisation can be performed in water (DissoCubes) or alternatively in non-aqueous media or water-reduced media (Nanopure). There is also a combination process of precipitation followed by a second high energy step, e.g. homogenisation (NANOEDGE). The result is a suspension of drug nanocrystals in a liquid, the so-called nanosuspension. Presented are the physical background of the diminution process, effects of production parameters (power density, number of homogenisation cycles) on crystal size, clinical batch production and scaling up of the production. As an important point the transfer of the liquid nanosuspensions to patient convenient oral dosage forms such as tablets and capsules is described.

Keywords: Drug nanocrystals; DissoCubes; Nanopure; Poorly soluble drugs; Bioavailability; High pressure homogenisation

1. Introduction

The number of drugs coming from synthesis and being poorly soluble is steadily increasing. At present about 40% of the drugs in the development pipelines and approximately 60% of the drugs coming directly from synthesis are poorly soluble [1]. This increasing number of poorly soluble drugs requires innovative formulation approaches to reach a sufficiently high bioavailability after oral administration or at least to make available intravenously injectible forms. There is quite a number of formulation approaches for drugs being poorly soluble in water, e.g. the use of solvent mixtures, cyclodextrines [2] or o/w emulsions for intravenous administration [3]. The principle limitation of all these approaches is that the drug needs to possess certain physicochemical properties (e.g. solubility in oils) or to 'fit' to the solubilising principle (e.g. having the right molecular size to fit into the cyclodextrine ring). These formulation approaches were of limited success as clearly demonstrated by the relatively low number of products on the market being based on such technologies. For example, there are only three main o/w emulsion products on the market with the drugs Diazepam, Etomidate and Propofol [4]. It would be much more elegant to have one universal formulation approach to process any poorly soluble drug. This is especially of interest for drugs being poorly soluble in aqueous media and simultaneously in organic media thus excluding all formulation approaches involving any solvent mixture. Especially, this group of poorly soluble drugs is increasing thus excluding many previous formulation approaches.

A meanwhile classical formulation approach for such poorly soluble drugs is micronisation, that means transfer of the coarse drug powder to an ultrafine powder with a mean particle size being typically in the range of 2–5 μm, size distributions normally range from approximately 0.1 to 25 μm [5,6]. It should be pointed out that only a negligible fraction of the population is below 1 μm. Micronisation is

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doi:10.1016/j.ejpb.2005.05.009

a very simple technology (e.g. by jet milling or wet milling). The principle was to increase the dissolution velocity by enlarging the surface area of the drug powder. To sum up, micronisation was or is a technology for case II drugs of the biopharmaceutical classification system (BCS), i.e. drugs having a good permeability but a low oral bioavailability due to their poor solubility and low dissolution velocity. Nowadays, many of the new drugs exhibit such a low solubility that micronisation does not lead to a sufficiently high bioavailability. Consequently, the next step was taken to move from micronisation to nanonisation, that means producing drug nanocrystals. By definition drug nanocrystals are nanoparticles being composed of 100% drug without any matrix material, according to the definition of nanoparticles the mean particle size is below 1 μm (i.e. in the nanometre range, typically somewhere between 200 and 500 nm).

There are several production techniques to produce drug nanocrystals. Basically, one can differentiate between top down and bottom up technologies which are reviewed briefly in this publication. Typically, the drug nanocrystals are generated in a liquid dispersion medium (e.g. by precipitation or a disintegration process). The obtained product from this process is a suspension of drug nanocrystals in a liquid stabilised by a surfactant or polymer (so-called 'nanosuspension'). In contrast to micronised powders the drug nanocrystals can be administered using very different administration routes. Oral administration is possible as a suspension. More patient convenient dosage forms can be produced by transferring the liquid nanosuspensions to solid dosage forms, i.e. tablets or pellets or granulate containing capsules. In addition, because of their small size the nanosuspensions can be injected parenterally, especially intravenously. Intravenous injection leads 'per definition' to a 100% bioavailability.

This paper focuses on the production of drug nanocrystals by high pressure homogenisation (top down technology), reviews their special properties, and discusses the potential final formulations for the patient including industrial aspects such as large scale production and regulatory issues.

2. Brief overview of existing technologies to produce drug nanocrystals

The existing technologies can be divided into the so-called 'bottom up' and the 'top down' technologies. The bottom up technologies start from the molecules which are dissolved and precipitate them by adding the solvent to a non-solvent. The top down technologies are disintegration methods, that means various types of wet milling.

Examples for precipitation techniques are the hydrosols [7–9] developed by Sucker (company Sandoz, nowadays Novartis), the product Nanomorph by the company Soliqs/Abbott (previously Knoll/BASF) and a number of other precipitation techniques [10–12] differing in precipitation details such as use of certain stabilisers (e.g. B.W. Müller/Kiel, Germany) [13]. Basically, the drug is dissolved in a solvent and this solution is added to a non-solvent. Addition of the solvent to the non-solvent is necessary to yield a very fine product by passing the Ostwald Mier area fast [14]. In the case of Nanomorph, amorphous drug nanocrystals are produced to further enhance dissolution velocity and solubility. The basic advantage of precipitation techniques is that they use relatively simple, low cost equipment. Scaling up is relatively easy possible by using static blenders (e.g. from the company Sulzer Chemtech) or micromixers (Institut für Mikrotechnik Mainz/Germany) [15]. The use of a static blender maintains practically the precipitation conditions in a beaker on lab scale, stirring and mixing problems potentially occurring when moving from lab scale to a large product container can be a priori avoided. However, there are basic problems associated with precipitation techniques. The particles produced need to retain their size after precipitation, particle growth to microcrystals needs to be avoided. In case a special crystalline state is given to the particle matrix (amorphous), this state needs to be maintained during the shelf life of the product to avoid a decrease in oral bioavailability.

To sum up, the bottom up techniques are not really widely used for drug nanocrystal production. Nowadays, the top down technologies of various milling techniques are more frequently used.

There are two basic disintegration technologies for drug nanocrystals:

1. Pearl/ball milling
2. High pressure homogenisation with different homogeniser types/homogenisation principles

In pearl milling, the drug macrosuspension is filled into a milling container containing milling pearls from, e.g. glass, zircon oxide or special polymers such as hard polystyrene derivatives. The pearls are moved by a stirrer, the drug is ground to nanocrystals in between the pearls. This is the basic technology developed by G. Liversidge and co-workers and nowadays used by the company Nanosystems (presently owned by élan). First products on the market are Rapamune and Emend, launched in 2002 and 2003, respectively. For reasons of convenience for the patient, the aqueous nanosuspensions have to be transferred to tablets. A general problem of pearl mills is potential erosion of material from the milling pearls leading to product contamination [16]. Nanosystems is using a polymer as pearl material to minimise erosion. The point of product contamination by erosion seems to be a hot topic. It is difficult to find definite numbers in the literature, in most cases figures are only given in oral presentations or discussions. The numbers range from 0.05 ppm which would be unproblematic to 70 ppm causing definitely problems. This can be summarised that there is no absolute

figure because the erosion depends on the hardness of the drug and the milling material and also the milling time required (e.g. hours or up to several days). Scaling up with pearl mills is possible; however, there is a certain limitation in the size of the mill due to its weight. Up to about 2/3 of the mill volume are the pearls leading to a heavy weight of the machinery thus limiting the maximum batch size. The batch size can be increased above the void volume (volume in between the hexagonal packaging of the pearls) using a mill with suspension circulation. The suspension is contained in a product container and continuously pumped through the mill in a circle. This increases the batch size but of course also the milling time because the required total exposure time of the drug particles per mass unit to the milling material remains unchanged.

The second most frequently used disintegration method is milling by high pressure homogenisation. The two homogenisation principles/homogeniser types applied are:

1. Microfluidisation (Microfluidics, Inc.)
2. Piston-gap homogenisers (e.g. APV Gaulin, Avestin, etc.)

Microfluidisation is a jet stream principle, the suspension is accelerated and passes with a high velocity an especially designed homogenisation chamber. In the 'Z' type chamber, the suspension changes a few times the direction of its flow leading to particle collision and shear forces. In the second type of chamber, the 'Y'-type, the suspension stream is divided into two streams which then collide frontally. The microfluidisation technique for drug nanocrystal production [17] has been pursued by the Canadian company Research Triangle Pharmaceuticals (RTP) (meanwhile acquired by SkyePharma PLC). A disadvantage of the technology is the sometimes high number of passes through the microfluidiser, examples in the various patents describe up to 75 passes. This is not very production friendly. In addition, from our experiences, the product obtained by microfluidisation can contain a relatively large fraction of microparticles (especially in the case of hard drugs) thus losing the special benefits of a real homogeneous drug nanocrystal suspension (Section 3.4).

In the knowledge of the potential problems associated with pearl/ball milling and the use of the microfluidisation principle, as an alternative a drug nanocrystal technology based on piston-gap homogenisers was developed in the middle of the 1990s. A first technology was based on homogenisation of particles in pure water [18], the trademark of the product is DissoCubes (trade name nowadays owned by SkyePharma). At the turn of the millennium, the second generation technology was developed, that means homogenisation of drug particles in non-aqueous media or in dispersion media with a reduced water content (i.e. mixtures of water with water-miscible liquids such as water–PEG or water–glycerol (e.g. isotonic suspensions for i.v. injections)) [19]. Registered trade name by the company PharmaSol GmbH/Berlin is Nanopure® (pure nanocrystals). Precipitation is the traditional approach to produce nanosized drug material, but having the problem of potential growth of drug nanocrystals to drug microcrystals. The company Baxter introduced a combination technology called NANOEDGE. Precipitation is followed by a second high energy step, typical high pressure homogenisation. Table 1 gives an overview of various technologies and patents applications on which they are based [20]. This paper reviews these piston-gap homogenisation techniques, highlights the differences with regard to the physics, covers scaling up as pre-requisite for the introduction of a product to the market and discusses briefly potential final formulations for the patient.

Table 1
Overview of the technologies and patents/patent applications on which the various homogenisation processes are based

Nanocrystal	Company	Patent/patent application examples
Hydrosol	Novartis (prev. Sandoz)	GB 22 69 536 GB 22 00 048
Nanomorph™	Soligs/Abbott	D 19637517
Nanocrystal™	élan Nanosystems	US 5,145,684
Dissocubes®	SkyePharma	US 5,858,410
Nanopure	PharmaSol	PCT/EP00/0635
NANOEDGE™	Baxter	US 6,884,436

3. High pressure homogenisation (piston-gap) for drug nanosuspension production

3.1. Homogenisation in water (DissoCubes)

Many years cavitation was considered as the most important effect to diminute particles in a piston-gap homogeniser. In this homogeniser types, the dispersion (emulsion or suspension) passes a very thin gap with an extremely high velocity. Prior to entering the gap, the suspension is contained in a cylinder with a relatively large diameter compared to the width of the following gap. In the APV LAB 40, the diameter of the cylinder is about 3 cm, it narrows to about roughly 25 μm (varies with applied pressure) when the suspension enters this homogenisation region (Fig. 1).

According to the law by Bernoulli, the flow volume of liquid in a closed system per cross-section is constant. That means the reduction in the diameter leads to a tremendous increase in the dynamic pressure and simultaneously a decrease of the static pressure when the liquid is in the homogeniser gap. A liquid boils when its vapour pressure is equal to the air/static pressure of the environment. In the gap the static pressure drops below the vapour pressure of the liquid at room temperature. Consequently, the liquid starts boiling, forms gas bubbles which implode after leaving the homogenisation gap and being again under normal air

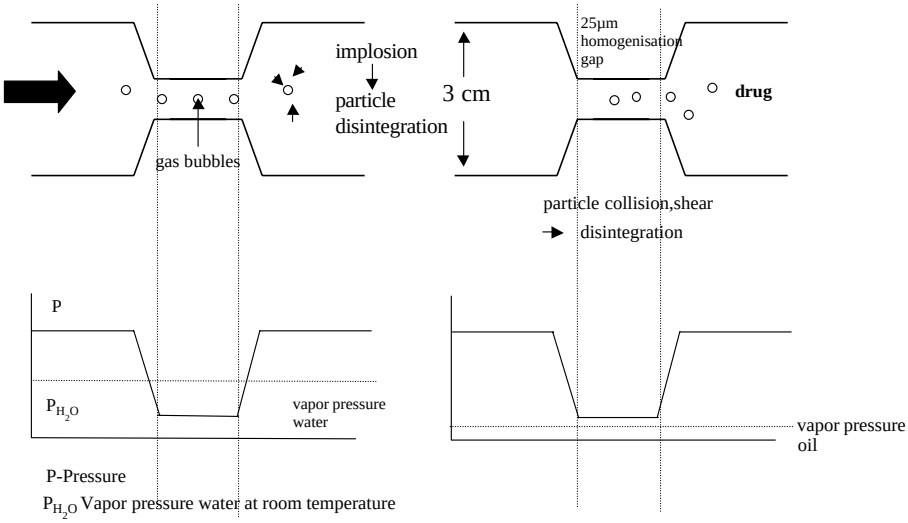

Fig. 1. Change of the diameter of the streaming dispersion in a piston-gap homogeniser from the cylinder containing the bulk suspension to the narrow homogenisation gap. The actual range of the static pressure as a function of the location inside the homogeniser is given in the diagrams below (left: situation for homogenisation in water, right: homogenisation in water-mixtures or water-free media).

pressure conditions. For this first technology DissoCubes, the cavitation was considered as the determining factor outlined in the respective patent [21].

As outlined in Section 3.3, it is important to have a pure drug nanocrystal suspension with low or very little presence of drug microparticles. To fully benefit from the increase in particle size by diminution to nanocrystals, it is also important to have a mean diameter below 1 µm. Independent on the actual drug nanocrystal size, at least the size should be homogeneous (as achieved with homogenisers) to avoid physical destabilisation. The size of the drug nanocrystals which can be achieved depends mainly on:

1. Power density of the homogeniser
2. Number of homogenisation cycles
3. Temperature

During a milling process, the particles/crystals break preferentially at weak points, i.e. imperfections. With decreasing particle size, the number of imperfections is getting less and less, that means the crystals remaining are becoming more and more perfect. Thus, the force required to break the crystals increases with decreasing particle size. If the force (power density) in the homogeniser is equal to the interaction forces in the crystal, the particles will not further diminute, even when additional homogenisation cycles are applied. That means the maximum dispersity at the given power density/homogenisation pressure is reached. Therefore, in general, to obtain a higher dispersity (smaller sizes) the homogenisation pressure needs to be increased, e.g. from 500 to 1000 or 1500 bar.

It should be noted that there is no linear relationship between decrease in size and increase in pressure, that means increasing the pressure stepwise by 500 bar will not lead to stepwise decreases of size in a linear relationship. Experiments with high pressures up to 4000 bar have shown that above a certain pressure only a relatively small further decrease in size could be achieved [22]. This can be explained that the crystals are getting more and more perfect, that means the force or energy required to break the crystals seems to increase rather exponentially. From this, for a drug particle with a given perfection of the crystal structure, only a certain small size can be achieved when applying production conditions which are realistic, i.e. can be applied in pharmaceutical production lines.

Fig. 2 shows the decrease in the mean diameter of the bulk population of a nanosuspension as a function of homogenisation cycles (size measured by photon correlation spectroscopy—PCS) [23]. PCS has a measuring size range of approximately 3 nm–3 µm, which means remaining drug particles above 3 µm, will not be detected. Therefore, it is necessary to characterise the nanosuspension additionally by laser diffractometry (LD). LD yields a volume distribution. A volume distribution is very sensitive towards the presence of even a few but relatively large particles (remaining large drug crystals or occurring aggregates of nanocrystals). Therefore, the diameters 90, 95 and 99% are sensitive markers for the presence or the

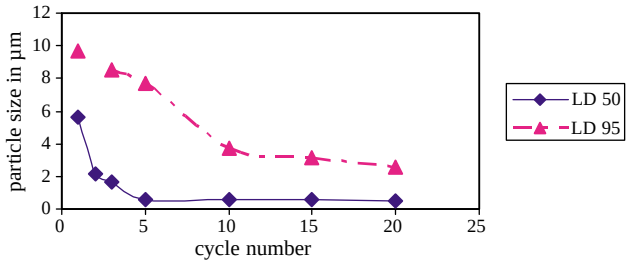

Fig. 2. Azodicarbonamide (ADA): decrease in the mean diameter in the bulk population (PCS data) and reduction in the number of large particles, characterised by the LD diameter 95%, as a function of cycle numbers.

disappearance of large particles during the homogenisation process. Fig. 2 shows that the maximum dispersity of the bulk population (PCS diameter) is reached after five homogenisation cycles, than the diameter stays practically unchanged. However, the diameter 95% further decreases reaching its lowest values at 15–20 cycles. This can be explained by a kind of two-step diminution process in the homogeniser. In the first step, the majority of the particles (bulk population) reaches relatively fast the maximum dispersity. Further homogenisation cycles have little effect on the mean diameter of the bulk, but reduce in the second step the width of the distribution by eliminating remaining few large crystals. Therefore—even when the mean diameter of the bulk population has reached its minimum and stays constant—additional homogenisation cycles are recommended. The improved homogeneity of the population further reduces potential effects of Ostwald ripening and is also sensible when preparing formulations for i.v. injection to avoid capillary blockade.

How can it be explained that certain larger particles escape the diminution process in the homogenisation gap? First off all, the lab scale homogeniser used for the particle production was a one punch machine, i.e. there are fluctuations in the homogenisation pressure. At lower pressure, diminution is less effective, some larger particles can 'survive' the homogenisation cycle. Furthermore, to achieve a uniform size distribution in a dispersion process (e.g. emulsification of oil) it is a pre-requisite that the power density distribution in the dissipation volume is uniform. In case there are zones of very high density, medium density and low density, the oil will only be dispersed to uniform droplets in the zone of medium power density. In the zone of too low density, relatively large droplets remain, leading to a polydisperse product. In the zone of very high power density, the uniform dispersed droplets acquire such a high kinetic energy, that they overcome the energy barrier according to the DLVO theory [24] and coalesce. The same phenomena can occur to some extent in the homogenisation gap of a piston-gap homogeniser. According to the turbulent flow profile in the gap, the zone of good dispersion capability is considered to be in the centre of the gap (highest streaming velocity). The streaming velocity decreases towards the wall of the homogenisation gap, thus resulting in lower disruptive forces leaving some large crystals to survive the passage of the gap (Fig. 3).

With increasing number of cycles, the probability that these larger particles pass also the zone of higher power density in the middle of the gap increases, thus finally also these particles are diminuted. The third effect discussed above for the process of emulsification of an oil, the coalescence in case of to high power density, can also be observed in the homogenisation process of nanosuspensions (i.e. aggregation in case of suspensions). Sometimes an increase in the mean diameter of the bulk population can be observed after 5–10 homogenisation cycles, followed again by a decrease. This can be explained that the ultrafine particles acquired a high kinetic energy, i.e. the homogenisation energy was not dissipitated in further breaking down of the particles (crystals are too perfect) and thus accelerating the particles. The surfactant selected to stabilise the suspension was not efficient enough and aggregates formed during this homogenisation cycle. In the next homogenisation cycles, these aggregates are again disrupted, leading again to a decrease in the mean diameter of the bulk population.

Another important determining factor for the final size of the drug nanocrystals is the hardness of the drugs. In case the drug is relatively soft such as paclitaxel, mean PCS diameters of the bulk population in the range of 250 nm will be obtained at typical production conditions of 1500 bar and 10–20 homogenisation cycles [25]. Diameters for other drugs reported are 493 nm for amphotericin B, 519 nm for itraconazole, 870 nm for carbamazepine, 660 nm for ketoconazole [26]. One relatively hard drug processed by now is azodicarbonamide (ADA). Depending on the production conditions, a bulk population of around 800 nm was found [27], but by modification of the process also diameters around 500–600 nm could be obtained (Fig. 2).

The nature and concentration of the stabiliser (surfactant or polymer) and potentially stabiliser mixture is a highly important factor for the fineness and physical long-term stability of emulsions produced by a dispersion process. This is different for drug nanosuspensions with regard to the fineness of the particles produced.

In case of emulsions, the stabiliser/surfactant reduces the interfacial tension between the oil and the water phase. Consequently, the energy for dispersing the oil droplets to a certain fineness decreases because the energy is equal to surface area × interfacial tension. At a given energy input (power density), finer droplets will be achieved in case of a high surface active stabiliser system (low interfacial tension). In the case of drug nanocrystals, the main energy has to be put in for breaking the crystal itself, that means overcoming the binding forces in the crystal lattice. In case of an oil droplet, there are very low binding forces (cohesion) between the oil molecules. Here, the main energy is required for the interfacial phenomenon. This is just opposite for drug crystals. These theoretical considerations are well in agreement with data reported in the literature, that the maximum dispersity/smallest particle size of nanosuspensions does not depend on nature and concentration of stabilisers [28]. It was also found that the shape of the nanocrystals does also not depend on the stabilisers. It purely depends on the type of crystalline structure in the starting material. For a given drug, the dimensions of the cuboid shape of the nanocrystals will remain the same, independent on the excipients used in the production process.

Erosion from the production equipment and subsequent contamination of the product is basically an important issue

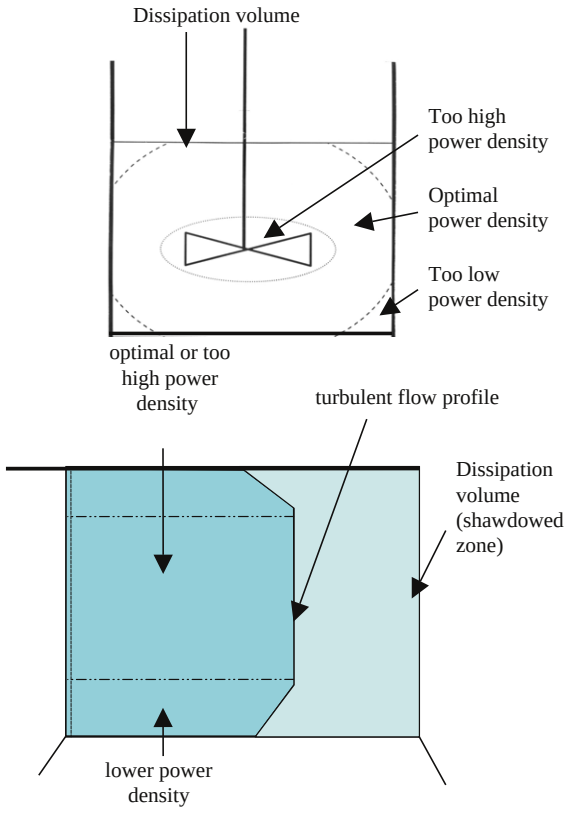

Fig. 3. Power density distribution in a stirred beaker with zones of optimal (medium), too low and too high power density, leading to a polydisperse dispersion result (upper) and comparable situation in the gap of a piston-gap homogeniser with highest energy zone in the centre and lower streaming velocity closed to the wall (lower).

in any production process. Homogenisers show a wearing at the homogenisation valve, due to cavitation and particle impact the initially smooth surface will show a macroscopically roughness after a certain time of usage. The potential erosion and contamination of the homogeniser was tested by using the drug RMKK98 and applying hard production conditions of 1500 bar and 20 homogenisation cycles. Iron as dominant metal in the steel was analysed by atomic absorption spectroscopy (AAS). The contamination found in nanosuspensions was 0.7 ppm, that means the other metals present in the steel are even in much lower concentrations [29].

To sum up, high pressure homogenisation of drug powders in water is a suitable method to produce drug nanosuspensions. Parameters determining the final dispersity are power density (homogenisation pressure), number of homogenisation cycles and hardness/softness of the drug. Stabilisers used have an effect on the long-term stability (avoidance of aggregates), but had no effect on maximum dispersity and no effect on the shape of the produced drug nanocrystals. Contamination from the production equipment is typically below 1 ppm, that means within a suitable range.

3.2. Homogenisation in water-free media and water mixtures (Nanopure)

For some administration routes or purposes, it is more convenient to have drug nanocrystals dispersed in non-aqueous media. Examples are drug nanocrystals in oils for filling of soft gelatine capsules or alternatively dispersed in liquid PEG 400 or 600. Highly chemically labile drugs could be produced in such non-aqueous media and diluted prior to, e.g. i.v. injection with water to yield an isotonic suspension (e.g. water–glycerol mixtures). For the transfer of liquid nanosuspensions into dry products it can also be desirable to have suspensions with a reduced water content and a more volatile dispersion medium, e.g. water–ethanol mixtures. As lined out above, the first patent of DissoCubes is based on the dominating role of cavitation in the homogenisation process. In contrast to water, oils and oily fatty acids have a very low vapour pressure at room temperature as clearly indicated by the much higher temperatures required for the boiling of oils. The boiling points of olive oil and oleic acid are 210 and 350 °C, respectively. As shown in Fig. 1, in the homogenisation gap the static pressure falls below the vapour pressure of water at room temperature, cavitation can develop. In case of dispersion media having a much lower vapour pressure than water, the drop in the static pressure is not sufficient enough to initiate cavitation or at least there will be very limited cavitation compared to water. Based on this, particle diminution should be not very efficient or distinctly less pronounced than in water.

Furthermore, based on the outlines above, cavitation and related particle diminution should be much more pronounced at elevated temperatures. At elevated temperatures, the vapour pressure of water is higher, that means when the static pressure decreases the boiling will be much more intense in case of a higher vapour pressure of the water. In patents covering the disintegration of polymeric material by high pressure homogenisation it is said that higher temperatures in the range of about 80 °C promote particle disintegration, e.g. as described for polymers [30]. However, for chemically labile pharmaceutical compounds, homogenisation at around 80 °C does not seem to be sensible [19].

When developing the second generation of drug nanocrystals Nanopure, just the opposite was done as described in the literature. Drug suspensions in non-aqueous media such as propylene glycol were homogenised. In addition to homogenisation at room temperature, the process was performed at 0 °C and well below freezing point (e.g. −20 °C), the so called ‘deep-freeze homogenisation’. The result was—against the teaching in the literature—that particle disintegration was similarly effective in non-aqueous media. This opens the perspective to prepare non-aqueous nanosuspensions for further direct processing, e.g. to oral dosage forms. In addition, homogenisation was performed below the freezing point

of water. From the theory, this should be even less effective because the vapour pressure of liquids decreases with decreasing temperature, thus leading to even less or no cavitation. Again, homogenisation results were comparable to homogenisation in water. This opens the perspective to process chemically labile substances at very mild conditions. Of course, homogenisation is a very fast process, temperature peaks occur only for milliseconds. Up to now, only with one compound, azodicarbonamide (ADA), a decomposition was observed during homogenisation at room temperature, visible by the formation of a foamy nanosuspension (formation of carbon dioxide due to decomposition of ADA) [27]. However, when using the second generation of the technology and homogenising ADA at 0 °C, no foamy nanosuspension was formed hence, the compound was obviously chemically stable.

The homogenisation in 'solid PEG' is a very interesting feature of the new technology for the formulation of oral dosage forms. To maintain the benefit of drug nanocrystals—the enhancement in bioavailability—it is of high importance that the drug nanocrystals are released from the oral dosage form as an ultrafine dispersion without aggregates. If aggregation occurs, the bioavailability decreases with increasing portion of aggregates. To achieve this, the drug nanocrystals should stay finely dispersed in the solid matrix of the oral dosage form, i.e. the tablet, pellet or capsule. A very elegant way to achieve this is homogenisation of drug powder in melted solid PEG, for example PEG 1000 (semi solid) or PEG 6000. Despite the fact that melted PEG is a low vapour pressure liquid, effective size reduction can be obtained, e.g. as shown for amphotericin B in Fig. 4. After five homogenisation cycles, the mean PCS diameter is around 600 nm and drops to about 200 nm after 25 cycles (Fig. 4).

After homogenisation, the PEG drug nanosuspension is cooled, the PEG recrystallises and fixes the drug nanocrystals inside the PEG matrix with a certain distance of the crystals to each other thus preventing crystal aggregation or

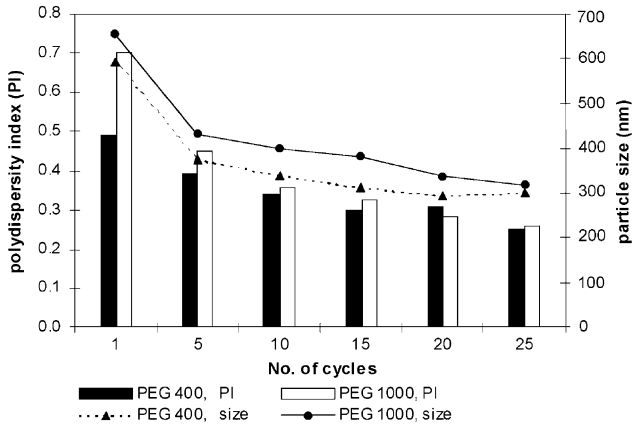

Fig. 4. PCS diameter and polydispersity index (measure of width of distribution) of amphotericin B powder homogenised in liquid PEG 400 and melted PEG 1000 (with permission after [31]).

Fig. 5. Amphotericin B nanosuspensions composed of liquid PEG 400 (left), as solid dispersion in solidified PEG 1000 (middle) and in form of ground solid PEG 1000 yielding a white powder (right) (with permission after [31]).

crystal growth. For the production of the final dosage form, the liquid PEG nanosuspensions (Fig. 5, left) can be filled into capsules. Fig. 5 shows the microscopic appearance of liquid PEG nanosuspension, solidified PEG 1000 nanosuspension and the powder produced by grinding (Fig. 5, right and Fig. 6).

For the drug nanocrystals in PEG being solid at room temperature (e.g. 1000, 6000) there are two ways of processing:

1. Filling of the hot melted nanosuspension directly into, e.g. hard gelatine capsules or HPMC capsules, the suspension solidifies inside the capsule or
2. Solidification of the PEG nanosuspension, grinding it to a white powder (Fig. 6) with subsequent filling of the powder into hard gelatine or HPMC capsules (Fig. 7).

The latter could have the advantage that the powder finely disperses faster in the GIT after dissolution of the capsule, thus accelerating dissolution of the PEG and redispersion of the drug nanocrystals.

Fig. 6. Appearance of solid PEG nanocrystal dispersion after milling to a fine white powder (with permission after [31]).

Fig. 7. Hard gelatine capsules directly filled with liquid hot PEG 1000 suspension (upper) and with granulated solidified PEG 1000 nanosuspension (lower) (with permission after [31]).

Fig. 7 shows the appearance of hard gelatine capsules after filling directly with the hot PEG 1000 (upper) nanosuspension or alternatively with the ground powder (lower). Of course, the drug nanosuspension product can also be used to produce tablets. Solid PEG is a normal excipient in tabletting, it can be admixed to a mixture for direct compression or to a one phasic granulate as outer phase for tablets produced in a granulation process.

Another elegant method to transfer drug nanosuspensions to a solid dosage form is the preparation of so called 'compounds'. Compounds are freely flowable powders, generally produced for mixtures for direct compression. DirectCompress is a technology for the production of such compounds. For example, the excipient lactose is dissolved and a non-water soluble polymer (e.g. ethylcellulose particles, Eudragit RSPO particles) is dispersed, simultaneously, in an aqueous nanosuspension, e.g. based on a water–ethanol mixture and this suspension is then spray-dried. The result is a freely flowable powder, optimal particle size is around 50–400 μm [32]. This compound can be used in a direct compression process to produce tablets. Alternatively, the product can be filled into hard gelatine or HPMC capsules. Fig. 8 shows the dissolution and redispersion of drug nanocrystals from such a DirectCompress compound [33]. The powder was put on the surface of water in a beaker and the re-dispersion recorded photographically as a function of time.

Amphotericin B drug nanocrystals were produced in glycerol–water mixtures [34]. Optimum dispersity with avoidance of aggregation was found in a glycerol–water mixture of the ratio 80:20 [34]. Basically, the reduction in water content increases the chemical stability. In this case, the glycerol nanosuspension was an intermediate product for the production of o/w amphotericin B emulsions for i.v. administration (so called SolEmuls technology [3,35,36]). Because of their small size, drug nanocrystals have excellent dissolution properties, thus allowing transferring amphotericin B from the crystal to the interfacial lecithin layer of an o/w emulsion in a very efficient way. Such an emulsion is a much more cost effective alternative to liposomal amphotericin B products such as AmBisome [37].

To sum up, drug nanocrystals can be similarly efficient produced in non-aqueous media or water mixtures as in pure water (i.e. more precise: surfactant solution). This Nanopure technology is especially suitable for the formulation of final oral dosage forms for the patients. Production of drug nanocrystals in mixtures of water with water-miscible liquids has process advantages, for example in case of spray-drying or lyophilisation. In addition it allows the production of isotonic drug nanosuspensions for i.v. administration in a one step production process (homogenisation in an isotonic glycerol–water mixture, intellectual property issue).

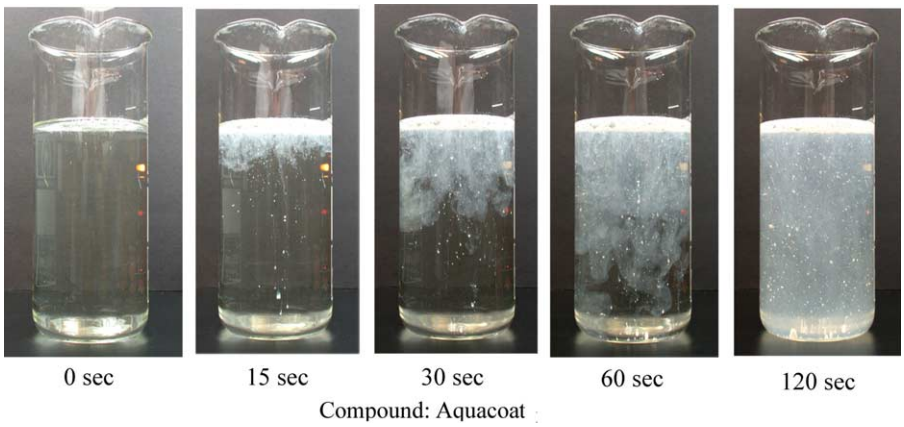

Fig. 8. Redispersion of a drug nanocrystal containing compound of DirectCompress® technology in water as a function of time.

3.3. Combination technology precipitation and homogenisation (NANOEDGE)

As outlined in 2., precipitated drug nanoparticles exhibit very often the tendency to continue crystal growth to the size of micrometre crystals. In addition, depending on the precipitation conditions the particles are completely amorphous, partially amorphous or completely crystalline. To ensure the long-term stability of the crystalline status, the easiest approach is to have particles in the low energy crystalline modification. Amorphous or partially amorphous particles bear the risk of re-crystallisation of this amorphous fraction followed by a decrease in bioavailability. Both problems, avoidance of further crystal growth and uncertainty of crystalline/amorphous state were solved by combining the precipitation with a second high energy addition step [12]. In the patent, it is shown that precipitated particles continued crystal growth if they are not undergoing the patent process step of high energy. In general, the precipitated particle suspension is subsequently homogenised which can basically preserve the size range of the particles obtained after the precipitation step. In addition, this 'annealing' process converts all precipitated particles to crystalline material. This removes all concerns about physical stability of amorphous material. The drug nanocrystals possess a definite crystalline state.

All basic principles outlined for the precipitation and for the high pressure homogenisation are also valid for this combination technology. That means this technology can only be applied for drugs which are at least soluble in one solvent, where a second miscible non-solvent is available for the precipitation process. Additionally, solvents of low toxicity should be employed to avoid any regulatory problems with solvent residues. Normally, the precipitation is performed in water using water-miscible solvents such as methanol, ethanol and isopropanol. Despite that solvents such as ethanol can be tolerated to a certain extent in liquid oral or parenteral formulations, it is desirable to remove it. The basic tendency is to have available ethanol-free formulations (e.g. ethanolic plant extracts are being continuously replaced by water based extracts). Removal of solvent is relatively easy on lab scale (e.g. by counter current flow), but is more problematic when producing larger batches of e.g. a tone. For the production of dry oral formulations or pellets, the solvents will be evaporated with the water anyway during the drying process of the tablets or of the pellets. It can be summarised that this combination technology seems to remove some problems of the precipitation process, but others such as the solvent remain. It addition, combination processes are more expensive than one step processes, especially when producing sterile parenteral products.

3.4. Special properties of drug nanocrystals

The rational of producing micronised drugs for oral administration is the enhancement in bioavailability for BCS class II drugs. The limiting step of oral absorption is the dissolution velocity. The dissolution velocity of micronised drug powders is enhanced by their enlarged surface area. The same effect, but much more pronounced, is valid for nanonised drug powders. The surface is even more enlarged, the dissolution velocity further enhanced.

Another important—but often neglected—aspect is the increase in saturation solubility. Textbook knowledge is that saturation solubility is a compound-specific constant only depending on the temperature and the properties of the dissolution medium. However, below a size of approximately 1–2 μm, the saturation solubility is also a function of the particle size. The theoretical background are the Kelvin equation [38], the Ostwald–Freundlich equation and the Prandtl equation [39]. For details it should be referred to the literature [40–42]. Briefly, according to the Kelvin equation, the vapour pressure above a curved surface is a function of the curvature. Droplets of a liquid below a certain size show an increased vapour pressure due to their strong curvature, that means the transition of molecules from the liquid phase of the droplet to the gas phase is accelerated. This principle is exploited when spray-drying liquids. The Kelvin equation describes the vapour pressure related to a transition of molecules from a liquid phase to a gas phase. This can be transferred to the dissolution of drugs from a solid particle phase to a liquid phase. That means the dissolution pressure corresponds to the vapour pressure in the Kelvin equation. The saturation solubility is an equilibrium between dissolving molecules (dissolution pressure) and re-crystallising molecules. Increasing the dissolution pressure shifts the equilibrium, the saturation solubility increases. The dependence of the saturation solubility on the particle size is also expressed in the Ostwald–Freundlich equation. The increase in saturation solubility has two effects:

1. Based on the Noyes–Whitney equation an increase in saturation solubility leads to an increase in dissolution velocity.
2. An increased saturation solubility in the lumen of the gut increases the concentration gradient between lumen and the blood, thus accelerating drug-diffusion, promoting absorption.

There is a third special feature of drug nanocrystals, the general adhesiveness of nanoparticles. Due to their large surface area, the nanoparticles tend to stick to surfaces. Based on physics this can be explained by the larger surface area providing more interactive forces between the particles and the surface, this interaction can be calculated [43]. The effect can be nicely demonstrated referring to daily life. Relatively large crystalline sugar does not stick that well to bakery compared to iced sugar. Iced sugar (fine particles)

can cover bakery in a very sticky layer (e.g. German Christmas speciality 'Dresdner Christstollen'). The adhesiveness of the particles to the gut wall after oral administration further enhances the bioavailability. The drug dissolves exactly at the place of its absorption. This process was found to be very reproducible, there is very little dependence on the nutritional state of the patients, i.e. between fed and fasted state [44].

To sum up, special features of drug nanocrystals are: the further enlargement in surface area by one dimension compared to micronised powders, the increase in saturation solubility, both leading to a distinctly increased dissolution velocity. Consequently, nanonisation is the ultimate universal formulation approach for drugs of BCS class II. In addition, due to their ultrafine character and adhesiveness, they further enhance oral bioavailability of drugs and reduce variability in bioavailability due to the reproducibility of there adhesion process to the gut wall.

3.5. Large scale production, scaling up issues

In general, scaling up encounters many problems, as the process parameters and process dimensions will change a lot within such a scaling up process. The lab scale machines used for the production of drug nanocrystals have a batch volume of about 3 ml (Avestin B-3) and 40 ml (Micron LAB 40, APV Homogenisers, Unna, Germany). Increasing the produced volume from 3 ml to a batch size of about half a ton (500 kg) means enlarging the production volume by a factor of approximately 165,000. Apart from having production capacities available, the first pre-requisite is to have available a qualified production unit to produce the batches for the clinical studies.

For the DissoCubes produced in water and the Nanopure nanocrystals produced in other dispersion media, the same equipment can be used. The principle of high pressure homogenisation is used in different areas ranging from food to pharma. High pressure homogenisation lines are accepted by the regulatory authorities for the production of emulsions for parenteral nutrition (e.g. Intralipid, Lipofundin). This is basically a very good starting position for establishing a new technology.

Another advantage is that the homogenisation valve is relatively similar or practically identical, at least the geometry, when moving from lab scale APV machines to production machines. From our scaling up experience, using larger volume machines proved even to be beneficial for the product quality. Less homogenisation cycles and less pressure were required, at the same time the product was even smaller in size and more homogeneous in size distribution (lower polydispersity index). This can be attributed to the fact that the production parameters can be much better controlled using the larger volume machines, e.g. the temperature. In addition, these machines are more effective because they are not single punch but multiple punch machines, there are distinctly less fluctuations in the homogenisation pressure compared to the lab machine LAB 40. In addition, they are equipped with two homogenisation valves in series. The second homogenisation valve immediately disrupts the aggregates potentially formed when the particles leave the first homogenisation valve. This second valve typically operates at 1/10 of the pressure of the first homogenisation valve (e.g. 500–50 bar).

A modified LAB 60 homogenisation unit was built for the production of technical batches [45]. The core of the unit is an LAB 60 homogeniser available from the shelf. It was modified this way that the capacity of the product containers was extended from half a litre to 10 l. The pre-suspension is produced in the first product container using a dissolver disk. The suspension is then passed through the LAB 60 homogenisation unit to a second product container. After complete passage of the suspension to product container 2, the suspension is then passed back to the first container by gravity. The next homogenisation cycle can follow. All the materials for this unit are pharma grade, the different parts of the machine such as product containers, pipes and also the homogenisation block can be separately temperature controlled if required. The machine can be sterilised with streaming steam. The dimensions were designed this way that it can be placed under a laminar air flow unit mounted at the ceiling of the production suite (Fig. 9).

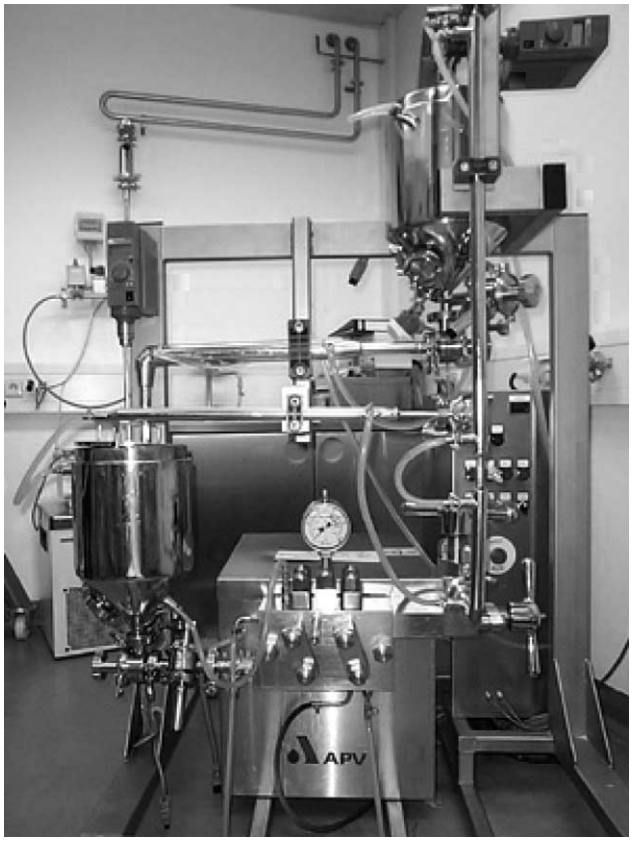

Fig. 9. LAB 60 production unit for aseptic production of drug nanosuspension.

For highly potent drugs, the clinical batch unit presented here can directly be a production unit. In case larger volumes of nanosuspensions are required, it is recommended to use a Rannie 118 with a capacity of 1 ton/h at the maximum applicable pressure of 1500 bar. Alternatively, an Avestin 1000 could be used providing a homogenisation capacity of 1000 l/h. The number of homogenisation cycles required for a fine drug nanosuspension is a minimum of 10, typically a maximum of 20. It would not be economical in a discontinuous batch production to pass the same batch 20 times through one homogeniser. The homogenisation equipment is off—the shelf equipment, at the same time it is relatively low cost. Therefore, it is recommended to place two or four homogenisers Rannie 118 in series. Assuming a capacity of 1000 kg/h in case of one homogeniser, the production of one tons nanosuspension would require 20 h (assuming 20 homogenisation cycles). In case of four homogenisers in series, this time would reduce to 5 h homogenisation time for 1000 kg of nanosuspension. Drug nanosuspensions can be produced with a 20% solid content without problems, in some cases we produced nanosuspensions with a 40% solid content. From this it is very likely that the batch size calculated as volume can be distinctly reduced by producing highly concentrated suspensions, which are diluted later on.

The large scale production lines described for aqueous and non-aqueous homogenisation can also be applied in principle for the Baxter combination technology NANOEDGE. In case of production of sterile products, there are two possible approaches:

1. Thermal sterilisation
2. Aseptic production.

Thermal sterilisation by autoclaving can be performed in case the drug is temperature resistant and the stabiliser combination is suitable. In general, it was found that identical to parenteral fat emulsions stabilisation of nanoparticles by lecithin can lead to dispersions being stable at autoclaving conditions of 121 °C, 2 bar. Nanosuspensions stabilised with steric stabilising polymers such as poloxamer 188 were observed to show flocculation. This was attributed to the dehydration of the polymer chains and reduced steric stabilisation efficiency. Of course these results cannot be generalised. In each case it depends on the affinity of the stabiliser to the drug nanocrystal surface and the resulting properties of the stabilising layer. Alternatively, gamma irradiation could be performed but this process is less desired in pharmaceutical production due to the necessary subsequent analytical procedures. An interesting alternative is aseptic production. Such a process can be exactly monitored and validated. The company Baxter realised an aseptic line for parenteral nanosuspension on larger scale. Such production line has also the advantage that the highly potent drugs such as cytotoxics can be processed. In addition, it should be born in mind that high pressure homogenisation process itself has a germ reducing effect. Not only the crystals but also the bacteria are ‘disintegrated’. The high pressure homogenisation is used in food industry to reduce the microbial level in food to prolong its shelf life.

3.6. Final formulations of drug nanosuspensions

Aqueous or non-aqueous drug nanosuspensions exhibit a physical long-term stability which in theory should be sufficient to place them on the market as liquid products. This might be suitable for certain groups of patients, e.g. children or elderly patients, but not for the ‘normal’ patient. In general, a dry oral dosage form is preferred, that means a tablet or a capsule. In case of drug nanosuspensions in pure water (DissoCubes) or in water containing mixtures (Nanopure) they can be used as granulation fluid in the granulation process for the production of tablets or alternatively as wetting agent for the extrusion mass to produce pellets. Spray-drying is also possible whereas water–ethanol mixtures will evaporate faster than pure water. The produced powders can then be used again for tablet or pellet production or alternatively be filled in hard gelatine or HPMC capsules.

The new feature of Nanopure is that drug nanocrystals can be produced in non-aqueous media such as oils or liquid/solid PEG which can directly be used for the filling of capsules. The dispersion of the crystals in oil promotes drug absorption exploiting the absorption enhancing effect of lipids [46–49]. Production of drug nanosuspensions in melted PEG which is solid at room temperature opens further perspectives. Direct filling of capsules with the hot nanosuspension is possible. Alternatively after solidification of the PEG, the drug nanocrystal containing mass can be ground (Fig. 6) and filled as powder in the capsules (Fig. 7).

To summarise, there are manifold different ways to transfer the drug nanocrystals to a final dry oral dosage form for the patient. With regard to parenteral products, the drug nanosuspensions can be used as they are, the shelf life of up to 3 years was shown for selected nanosuspensions [50]. Alternatively, lyophilised products can be offered to be reconstituted prior to administration.

4. Products on the market/in clinical phases

Looking at the time between invention of a technology and the first products on the market, this time period is very short for the drug nanocrystals. The liposomes were invented in 1968 by Bingham, the first pharmaceutical products appeared on the market at the beginning of the 1990s, that means approximately 20 years in between. The first drug nanocrystal patents were filled at the beginning of the ninties by the company Nanosystems (nowadays élan), the first product Rapamune was placed on the market in the year 2000 by the company Wyeth. The product is a tablet

containing 1 or 2 mg of sirolimus. At the same time there is a solution on the market. The tablet has the advantage of being more use-friendly than the solution. Compared to the solution, the tablet has 21% higher bioavailability. That means the drug nanocrystals perform even better than an orally applied solution, normally applied as optimised standard in bioavailability studies. To fully benefit from the bioavailability enhancement of drug nanocrystals, it is a prerequisite that the nanocrystals are released as an ultrafine, non-aggregated suspension from the oral dosage form. To achieve this, the drug nanocrystal concentration in a tablet has a certain upper limit. In case this limit is exceeded, particles are getting in contact and might fuse under the compression. For Rapamune it was beneficial that a very low drug nanocrystal amount had to be incorporated into the tablet, just 1–2 mg, the total tablet weight is approximately 360 mg. That means the drug nanocystal content is below 1% causing no formulation difficulties.

The second product Emend was already introduced in the following year 2001 by the company Merck. It is a capsule containing 125 mg of the drug aprepitant. The filling material of a capsule are pellets. A single dose of 125 mg is in relation to typical weights of oral dosage forms between 400 and 500 mg relatively high. It corresponds to approximately 30 and 25% of drug nanocrystals in the total dosage form. In case of such a higher content it might be beneficial to apply less forces during the production process, that means replacing compression by an extrusion procedure (i.e. producing pellets).

In the middle of the 1990s there was reluctance of pharmaceutical companies to employ the drug nanocrystal technology. At this stage it was a relatively hearing technology. A prerequisite for companies to use a technology is the availability of large scale production facilities. At the very beginning these facilities were unavailable. In addition, before moving to a novel technology one tries to employ technologies already existing in the company. In case of a successful formulation, large scale production technologies are available for these formulations approaches. The situation changed with the establishment of large scale production facilities and the 'pressure' created by an increasing number of poorly soluble compounds, i.e. the very low solubility, in both aqueous and organic media excluding the use of many traditional formulation approaches. Large scale production units for pearl milling were realised by circulating the suspension through the peral mill (élan), the company Baxter established an aseptic production line based on piston-gap homogenisers for the product platform NANOEDGE. This let to the acceleration of the development of formulations based on drug nanocrystals. Quite a number is meanwhile in clinical phases. Table 2 gives an overview.

5. Conclusion and perspectives

Drug nanocrystals can be considered as a universal formulation approach for poorly soluble drugs. The striking advantage is that the drug nanocrystals can be applied to various administration routes, that means oral but also parenteral, especially i.v. administration. I.v. administration leads to 100% bioavailability and allows pharmacological screening of any new chemical entity independent on its solubility properties. Other

Table 2
Overview of drugs being presently in different clinical phases after [20]

Drug	Indication	Drug delivery company	Pharma company	Route	Status
Paclitaxel	Anticancer	American BioScience	American Pharmaceutical Partners	Intravenous	Phase III
Undisclosed multiple	Anti-infective	Baxter NANOEDGE	Undisclosed	Oral Intravenous	Preclinical to Phase II
Undisclosed	Anticancer	Baxter NANOEDGE	Undisclosed	Intravenous Oral	Preclinical to Phase I
Rapamune	Immuno-suppressant	Elan Nanosystems	Wyeth	Oral	Marketed
Emend	Anti-emetic	Elan Nanosystems	Merck	Oral	Marketed
Cytokine inhibitor	Crohn's disease	Elan Nanosystems	Cytokine PharmaSciences	Oral	Phase II
Diagnostic agent	Imaging agent	Elan Nanosystems	Photogen	Intravenous	Phase I/II
Thymectacin	Anticancer	Elan Nanosystems	NEwBiotics/Ilex Oncology	Intravenous	Phase I/II
Fenofibrate	Lipid lowering	SkyePharma	Undisclosed	Oral	Phase I
Busulfan	Anticancer	SkyePharma	Supergen	Intrathecal	Phase I
Budesonide	Asthma	Elan Nanosystems	Sheffield Pharmaceuticals	Pulmonary	Phase I
Silver	Eczema atopic dermatitis	NUCRYST	Self-developed	Topical	Phase I
Calcium phosphate	Mucosal vaccine adjuvant for herpes	BioSante	Self-developed	Oral	Phase I
Insulin	Diabetes	BioSante	Self-developed	Oral	Phase I

administration routes not discussed in this article are dermal delivery to create supersaturated systems with high thermodynamic activity, ophthalmic administration to create systems with prolonged retention times, nasal administration to stick nanocrystals to the nasal mucosa, vaginal administration to create systems evenly spreading throughout the therapeutic area, and aerosols containing drug nanocrystals for pulmonary delivery. It appears also feasible to inject drug nanocrystal suspensions locally for treatment of tumours which cannot be removed by surgery. Very attractive options are the targeting of i.v. injected drug nanocrystal suspensions by using the concept of the PathFinder technology [51–53]. The drug nanocrystals are surface-modified this way that they preferentially adsorb the blood proteins for site specific localisation, for example in the brain or the bone marrow. Independent on the way of production, the drug nanocrystals have one big advantage: they are a simple system, simple to produce, simple to use. The more complicated a delivery system, the longer is the way to the patient and to the market. Simple approaches can be realised much faster as proven by the first nanocrystal products on the market. And a simple system does not mean necessarily that it is not smart. The smartness is the simplicity.

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