1 Introduction and aims of thesis

1.1 Introduction

The number of poorly soluble drugs is steadily increasing. About 40% of the drugs in the development pipe lines and appr. 60% of the drugs coming directly from chemical synthesis are poorly soluble. Consequently most of them exhibit a poor oral bioavailability because in general low saturation solubility is correlated with low dissolution velocity. If the dissolution velocity is too low, a sufficiently high blood level cannot be achieved (Biopharmaceutical Specification System (BCS) class II drugs). At present the number of new chemical entities (NCE) with a potential to be commercialised as drug is steadily decreasing. High numbers of compounds need to be synthesised before a lead compound is found. In this situation it is a big loss if an active compound cannot be introduced into the clinic because of low solubility and the lack of an efficient delivery system to make this drug sufficiently bioavailable. A smart delivery system can rescue a drug development project.

Consequently research focuses on the development of formulation approaches to overcome the oral bioavailability problems (Müller and Hildebrand 1998) There are so called “specific approaches”, that means the solubility of a molecule can be increased if it “matches” to the requirements of the delivery system (e.g. a molecule needs to fit into the cavity of a cyclodextrin ring). Ideal would be a universal formulation approach practically applicable to any drug. The drug nanocrystals are such a universal formulation approach. Nanomaterials exhibit special physical properties being different to the bulk material. The special properties of drug nanocrystals are increased saturation solubility and increased surface area, both leading to an increase in the dissolution velocity. Nanonisation is therefore the ultimate solution to increase the oral bioavailability in cases where the dissolution velocity is the rate limiting step. In addition the concentration gradient between lumen of the gut and the blood is increased. Chapter 2.2 provides an overview of the physical background of the nanocrystals, the different technologies of production available, large scale production, final formulation methods and the first marketed products.

The present drug nanocrystals on the market are “simple” systems, just the dissolution velocity is increased. However, there are also drugs which are poorly soluble because the dissolution velocity is low and simultaneously the permeation is low (BCS class IV). Low permeability can be caused by the efflux pumps present in the gastrointestinal drugs, e.g. p-

glycoprotein (Wiese and Seydel 2003). A typical example is cyclosporine. The challenge is to create “smarter” nanocrystals, which do not only dissolve fast but are combined with a technology to inhibit p-glycoprotein. When the development of the nanocrystals started (beginning of the 1990ies) limited knowledge and awareness (!) about this problem existed. Meanwhile the knowledge has increased, strategies for inhibition can be developed. Chapter 2.1 gives an overview. Ideal would be to combine the drug nanocrystals with a p-glycoprotein inhibition strategy, i.e. making “smart nanocrystals”.

Cyclosporine is on the market as a microemulsion, which shows a reduced variability compared to the first product Sandimmun introduced in 1983. However, the microemulsion shows an undesired plasma peak above 1000ng/ml being held responsible for nephrotoxicity. In addition there is still a certain variation in the bioavailability. An improved formulation could be nanocrystals providing similar fast release/dissolution as the microemulsion but avoiding the peak (e.g. by combination with traditional controlled release technology of pellets).

To successfully develop nanocrystals, a sound and fast sizing technology is the essential basis! Laser diffractometry is nowadays widely used, instrument manufacturers claim sizing ability down to the low nanometer range (40nm). However, size analysis in the nano range requires Mie theory, input of real and imaginary refractive index, which are in most cases not known. Now the question arises, how sound is laser diffractometer analysis in the nanometer range? Chapters 4.1-4.3 provide the background and highlight the problem. Before controlled development of a nanocrystals formulation can start, a valid and validated size analysis has to be available.

1.2 Aims of thesis

As outlined above there is still a need for a clearly improved oral formulation of cyclosporine. A first nanocrystal formulation developed by S. Runge (Runge 1998) did not show the theoretically expected increase in bioavailability. In contrast to other literature data about bioavailability increases by nanosuspensions – e.g. from 5 % to about 80 % in case of Danazol (Liversidge and Cundy 1995) – the cyclosporine nanosuspension by Runge completely failed (bioavailability almost zero). Therefore the **overall aim of the thesis** was:

The development of a physically optimised oral cyclosporine nanosuspension in vitro, with the potential to show the expected increase in bioavailability.

This cyclosporine nanosuspension should benefit from the scientifically already proven high reproducibility of the bioavailability of nanosuspensions in general, and the very little difference between fasted and non-fasted state (Liversidge and Cundy 1995).

This overall aim should be realised in four steps (sub aims):

First step:
Evaluation and validation of size analysis

A prerequisite for the development of optimised nanosuspensions is the availability of a precise size analysis method. Due to its fastness laser diffractometry (LD) is frequently used for crystal size determination to be able to handle the large sample numbers in such a development.

However, the analysis in the nanosized range requires the input of the real refractive index (RI) and the imaginary refractive index (IRI), parameters normally unknown. In the research group – but also by about estimated 90% of the scientists world-wide – simplified approaches were used for analysis. For example just using Fraunhofer analysis and hoping that the result would not be that far away from reality!

To be able to perform a sound development, in the first part of the thesis it should be evaluated to which extent the present LD analysis is valid at all, and - if it is not sound - to develop a better, reliable analytical LD procedure.

Second Step:
Evaluating the soundness of the existing screening procedures established and used by now for obtaining optimised nanosuspension

To minimise the work in a screening procedure, instead preparing 20 different nanosuspensions by homogenisation, a stock nanosuspension of 40g is generally prepared and then divided in 20 portions to which other surfactant solutions are admixed. This yields practically 20 different formulations by one production step.

However, nobody questioned by now, if this is a correct procedure or if artefacts might occur.

To be able to perform a sound development, apart from a proper size analysis, of course the soundness of the screening scheme needed to be evaluated.

Third step:

Coming up with a novel concept for an improved oral cyclosporine nanosuspension

The nanosuspension by Runge (dating back to 1996) showed an extremely poor bioavailability. The problem could definitely not just being solved by trying different other stabilisers without a new rational behind. The new rational to be tried in this thesis was to investigate if surfactants or steric stabilisers, being simultaneously a p-glycoprotein inhibitor, were able to efficiently physically stabilise cyclosporine drug nanosuspensions

Fourth step:

"Thinking out of the box" (in German: Querdenken) about other formulations than necessarily nanosuspensions or lipid nanoparticles

In the research group delivery problems are tried to being solved only by two approaches: drug nanocrystals and solid lipid nanoparticles. Of course these approaches narrow the horizon to look for other interesting alternatives.

One aim of this thesis was to think about something else than just using these two delivery systems. Look across the edge of the plate!

To summarise:

The thesis should critically question knowledge to be considered as solid by now, i.e. the routine procedures performed within the research group (but also many scientists world-wide), come up with improved solutions and additionally come up with a novel smart concept for drug nanocrystals of drugs of BCS class IV (e.g. cyclosporine), i.e. not showing just poor solubility but also poor bioavailability due to an efflux pump system (in this case p-glycoprotein).

Or just simply saying it in one sentence:

Question everything and create a novel nanosuspension concept to make a new dimensional step forward in nanocrystals formulation development.

2 Theoretical background

2.1 P-Glycoprotein and its influence on poor bioavailability

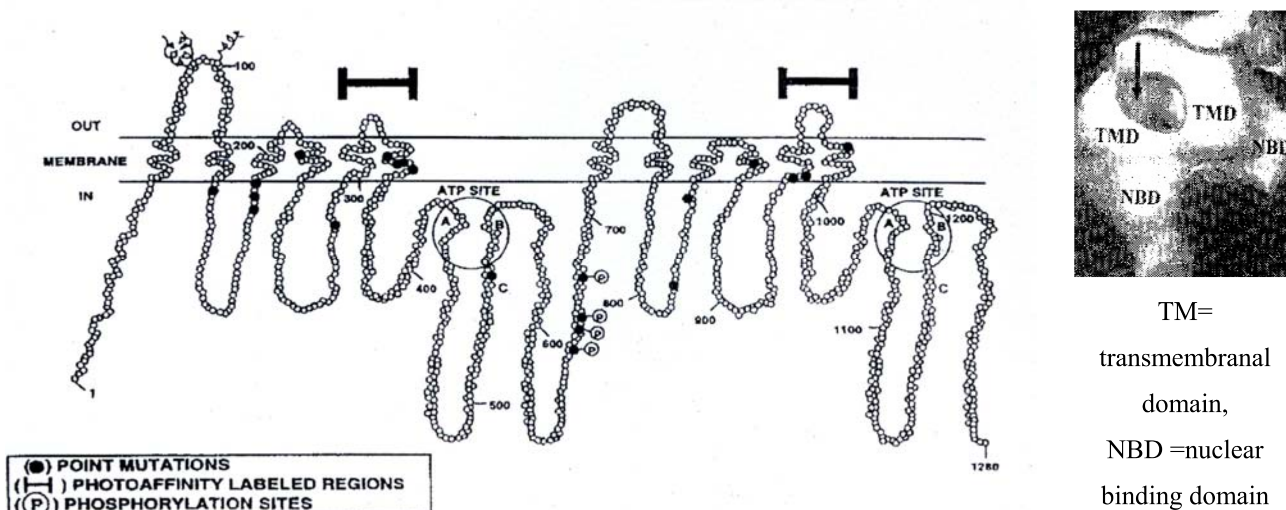
2.1.1 History

P-gp was first described in 1976 to be a transmembranal protein when it was isolated from cells that showed multi drug resistance. Because it was identified to alter the ***p***ermeation of drugs in cells, it was named the p-glycoprotein (Juliano and Ling 1976).

2.1.2 Characterisation

P-gp is a 170-kDa phosphorylated and glycosylated plasma membrane protein and belongs to the ***A***TP-***b***inding ***c***assette super family of ***transport*** proteins (ABC-transporter). All ABC-transporter consist of two transmembranal hydrophobic and two intracellular hydrophilic chains and transport a large amount of substrates, varying in structure, in an energy dependent way outside of the cell (Gottesman and Ambudkar 2001; Borst and Elferink 2002).

P-gp is 1280 amino acids long and consists of two homologous halves of 610 amino acids joined by a flexible linker region of 60 amino acids. Each half has an N-terminal hydrophobic domain containing six hydrophobic transmembranal domains followed by an intracellular hydrophilic domain containing a nucleotide-binding site (NBS). The two halves of the protein interact to form a single transporter, whereby domain 1 is located next to membrane 12, so when viewed from above the plasma membrane, is donut shaped, with 6-fold symmetry. It has a diameter of about 10 nm and a large central pore of about 5 nm in diameter and the thickness in the plane of the plasma membrane is about 8nm. Since the thickness of the plasma membrane lipid bilayer is about 4nm, about half of the molecule is within the plasma membrane. The central pore is closed at the inner (cytoplasmatic) end, forming an aqueous chamber within the membrane that is open to the extracellular medium. (Figure 2-1). There is an opening from this chamber to the lipid phase. Two lobes of about 3 nm each are exposed at the cytoplasm end and probably correspond to the nucleotide binding domains. (Gottesman and Pastan 1988; Lelong et al. 1992; Ambudkar et al. 1999; Gottesman and Ambudkar 2001). The nucleotide binding sites are non substrate binding, but change there conformation while substrate binding (Loo et al. 2003).



TM= transmembranal domain, NBD =nuclear binding domain

Figure 2-1: Right: Structure of p-gp (2D) after (Chin et al. 1993) and left: donut shape (3D) from of p-gp (Birds perspective) after (Rosenberg et al. 1997)

2.1.3 Mechanism of action

Several models for p-gp function have been proposed. The whole mechanism of action is not fully understood, but the most reasonable mechanism, which was first suggested by Gottesman and Pastan, is the hydrophobic vacuum cleaner model (Gottesman and Pastan 1993). This model is illustrated in Figure 2-2 and briefly described below.

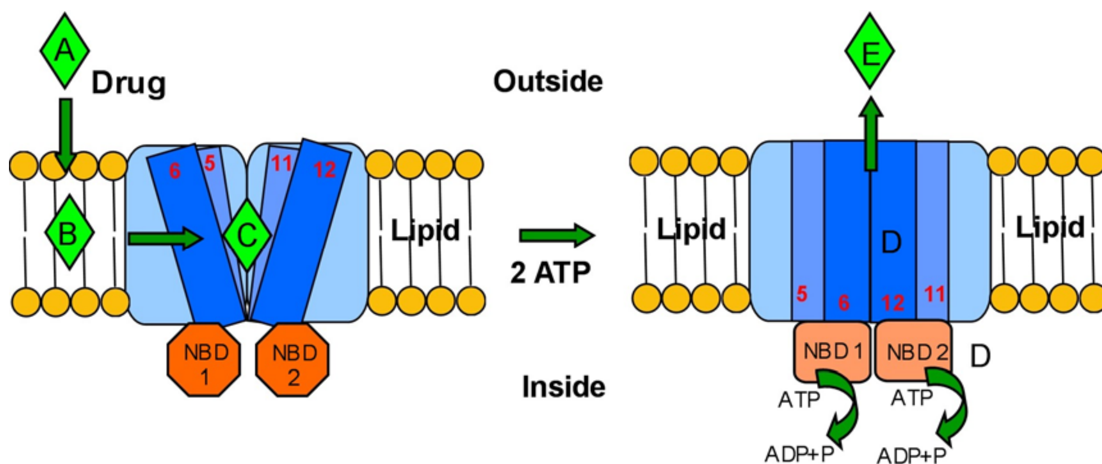


Figure 2-2: Mechanism of Drug transport modified after(Loo and Clarke 1999)
5, 6, 11, 12 are the involved transmembranal domains (TMDs), NBD 1 and NBD 2 are the nucleotide binding sites. The mechanism is explained in the text below.

Drug substrate (A) in the extracellular fluid diffuses first into the lipid bilayer (B) and then into the drug-binding site of p-gp. The drug-binding pocket is located in the interface between the two halves of the molecule and is closely associated with the transmembranal domains (TMDs) 6 and 12. After a drug enters the lipid bilayer it interacts with specific residues in the drug-binding pocket(C). ATP hydrolysis at both of the nuclear domain binding domains

(NBD) follows and leads to and conformational change in the TMDs. This causes TMD 6 and 12 to come very close together, which leads to the occlusion of the drug-binding site (D) and the simultaneous release of the drug (E).
The function is also influenced by the properties of the membrane, hence it depends of the consistency of the lipid bilayer as well as on properties such as liquid crystalline structure, which is influenced themselves by temperature, water content and ion concentration (Lu et al. 2001).

2.1.4 E*xpression*

P-glycoprotein is encoded by the human MDR1 gene. Rodents encode it on two genes mdr 1a and mdr 1b. The mechanism of expression is clear, but investigations of the influences by substrates, hormones and enzymes will be the aim of research for the next years. P-glycoprotein was found to be expressed in membranes of several tissues and cells. It is always located at the apical side, dividing tissue from the blood. P-gp is found in the liver, in hepatocytes on the biliary canalicular front and on the apical (luminal or central canal) surface of epithelial cells in small biliary ductules. In the pancreas, it is found on the apical surface of the epithelial cells of the small ductules, but not larger pancreatic ducts, also in the kidney on the apical surface of epithelial cells of the proximal tubules, in the adrenal gland in high levels on the surface of cells in both the medulla and the cortex. P-gp is also found in the sub-apical surface of the epithelium of the choroid plexus of the brain (which forms the blood-cerebrospinal fluid (CSF) barrier) as well as the luminal surface of the endothelium of blood capillaries of the brain (the blood-brain barrier) (Wijnholds et al. 2000). P-gp is involved in pregnancy and is expressed at extremely high levels in the gravid compared with the non-gravid uterus. This expression is specifically localised at the luminal surface of the secretory epithelial cells of the endometrium. (Arceci 1998). Cancer cells where found to over express p-gp. The expression of p-gp is manifold, for more detailes it is referred to the literature citied.
Most interestingly p-gp is also found in the colon and the jejunum: in high concentrations on the apical surfaces of superficial columnar epithelial cells; In the small intestines regional variation in p-gp expression has been demonstrated, with moderate p-gp expression found in the duodenum and jejunum and maximal expression in the ileum (Yumoto et al. 1999).

2.1.5 Substrates of p-gp

P-gp is a multi drug transporter. Substrates chemicals transported by p-gp have very diverse structures, which only share the properties of being hydrophobic amphipathic molecules

(molecules having two sides with characteristically different properties) that are not negatively charged, and may be 200-1800 Da in size. Nevertheless, under different conditions it may transport also hydrophilic and negatively charged compounds e.g. methotrexate. Beside many drugs, including cancer drugs, immunosuppressive drugs, antihistamines, cardiac drugs, lipid lowering agents, as well as antibiotics, it also transports physiological substances, such as steroids, phospholipids, hormones as well as enzymes (Ambudkar et al. 1999).

2.1.6 Modulators of transport function of p-gp

There is also a large amount of substrates which influences the transport function of p-gp. Inhibition or activation may take place at different stages of the transport. Inhibition of drug transport could potentially result from the blockage of the specific recognition of the substrate, binding of ATP, ATP hydrolysis, or coupling of ATP hydrolysis to translocation of the substrate. Most of the known reversing agents block drug transport by acting as competitive or non-competitive inhibitors (Garrigos et al. 1997) and by binding either to drug interaction sites or to other modulator binding sites leading to allosteric changes (Dey et al. 1997). The transport is an active ATP dependent mechanism which is catalysed by ATPase. Another possibility to influence the transport function can be obtained by changing the environment of the cell or the bilayer properties (Pajeva and Wiese 2002).

2.1.7 Inhibition of p-gp for increase of bioavailability

There is moot that the co-administration of p-glycoprotein inhibitors increases the bioavailability for drugs being substrates for p-gp. Therefore, the interest is the addition or co-administration of p-gp inhibitory compounds, when formulating drugs having a low bioavailability caused by this efflux pump. The inhibition of p-glycoprotein is possible and many compounds were identified to inhibit p-gp, leading to an increase in bioavailability of substances, being transported by it (Englund et al. 2004). However, co-administration of potent drugs acting as inhibitors can lead to adverse side effects and undesired phamacodynamic effects by the inhibitor themselves. Therefore, only substances without any pharmacodynamic effect are subjects of interest. Those compounds can be excipients with GRAS status or other additives without pharmacodynamic properties, e.g. food. In deed during the last years a broad variety of such substances could be identified to inhibit p-gp,

Table 2-1: Selected compounds which inhibit p-glycoprotein

Compound	Reference
D-α-Tocopheryl-polyethylenglycol-succinate (TPGS)	(Dintaman and Silverman 1999; Bittner et al. 2002; Johnson et al. 2002)
Pluronics (i.e. Poloxamers)	(Johnson et al. 2002; Kabanov et al. 2003)
Tween 80	(Rege et al. 2002; Szu-Wen Wang 2004)
Tagat TO	(Rege et al. 2002; Szu-Wen Wang 2004)
Chremophor EL	(Rege et al. 2002; Szu-Wen Wang 2004)
peppermint oil	(Wacher et al. 2002)
curcumin from curcuma	(Anuchapreeda et al. 2002)
piperine from black pepper	(Bhardwaj et al. 2002)
falvonoids, consistent in many coloured vegetables (e.g. pepper or broccoli)	(Nguyen et al. 2003)

without being a potent drug itself (Szu-Wen Wang 2004). Some of these compounds were selected and are shown in Table 2-1.

2.2 Nanosuspensions – the formulation approach for overcoming poor solubility problems of drugs

2.2.1 Definition

A nanosuspension is as a liquid dispersion consisting of solid drug nanoparticles (nanocrystals). By definition drug nanocrystals are nanoparticles being composed of 100% drug without any matrix material, with a mean diameter below 1000nm. The dispersion medium can be water, aqueous solutions or non-aqueous media. Surfactants and/or polymeric stabilisers are used for the stabilisation of these systems.

2.2.2 Properties of nanosuspensions

Nanonisation of drug powders increases the surface of the particles, leading to an increase of the dissolution velocity. Another important 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 1-2µm, the saturation solubility is also a function of the particle size. The theoretical background are the Kelvin equation (Simonelli 1970), the Ostwald-Freundlich equation and the Prandtl equation (Mosharraf 1995). For details it should be referred to the literature (Jacobs 2000; Müller 2000; Müller 2003). 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. Hence, 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.

The third special feature of drug nanocrystals is 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 (Stieß 1995). The effect can be 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 (Liversidge 1996).

To sum up, special features of drug nanocrystals are enlargement in surface area, the increase in saturation solubility, both leading to a distinctly increased dissolution velocity and subsequently increased bioavailability. 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 their adhesion process to the gut wall. Consequently, nanonisation is the ultimate universal formulation approach for poorly soluble drugs, where the rate limiting step of oral absorption is the dissolution velocity.

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 appr. 60% of the drugs

coming directly from synthesis are poorly soluble (Merisko- Liversidge 2002). 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 injectable forms. There is quite a number of formulation approaches for drugs being poorly soluble in water, e. g. the use of solvent mixtures, cyclodextrines (Frömming 1993) or o/w emulsions for intravenous administration (Müller 2001). The principle limitation of all these approaches is that the drug needs to possess certain physico-chemical 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 (Schmitt 1998). 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 mixtures. 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. Micronisation means the transfer of coarse drug powder into an ultrafine powder with a mean particle size ranging from 2-5 µm. Size distributions normally range from approximately 0.1 to 25 µm (Müller 1995) and only a negligible fraction of the population is below 1 µm. Nowadays many of the new drugs exhibit such a low solubility that micronisation does not lead to a sufficiently high bioavailability. Consequently only nanonisation, hence the production of drug nanocrystals is a suitable formulation principle of those drugs.

2.2.3 Production of nanosuspensions - overview of existing technologies

There are several production techniques to produce drug nanocrystals. In principle, one differentiates between top down and bottom up technologies, which are reviewed in here briefly. Typically, 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, 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 into solid dosage forms, i.e. tablets, 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.

The existing technologies are the so-called “bottom up” and the “top down” technologies. The “bottom up” technologies start from the molecules, which are dissolved and precipitated then by adding the solvent to a non-solvent. The top down technologies are disintegration methods, e.g. various types of wet milling.

Examples for precipitation techniques are the hydrosols (List 1988; Gassmann 1994; Sucker 1994) which were developed by Sucker (company Sandoz, nowadays Novartis). Other products based on precipitation are Nanomorph by the company Soliqs/Abbott (previously Knoll/BASF) and a number of other precipitation techniques (Violante 1991; Thies and Muller 1998; Kipp 2003) differing in precipitation details such as use of certain stabilisers (e.g. B.W. Müller/Kiel, Germany) (Rasenack and Muller 2002). Basically hydrosols are produced by dissolving the drug in a solvent and adding this solution 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 (List 1982). 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) (Müller 2001). The use of a static blender practically maintains 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, a priori can be 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 the production of drug nanocrystals. To my knowledge, no commercial product based on these technologies is on the pharmaceutical market. 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 and/or 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 followed by Tricor (2004) and Megace ES (2005). For reasons of convenience for the patient, the aqueous nanosuspensions have to be transferred to tablets. A general problem of pearl mills is a potential erosion of material from the milling pearls leading to product contamination (Buchmann 1996). 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 of the 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 correspond to the weight of 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 existing 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 (Parikh 1999) 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, 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.
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 nineties. A first technology was based on homogenisation of particles in pure water (Müller 1999), 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. In comparison to the older method homogenisation of drug particles takes place 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)) (Müller 2000). The registered trade name by the company PharmaSol GmbH/Berlin is Nanopure® (pure nanocrystals). Precipitation is the traditional approach to produce nanosised 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 2-2 gives an overview of the different technologies and patents/ patent applications on which they are based (Rabinow 2004).

Table 2-2: Overview of technologies and selected examples of 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™	ēlan Nanosystems	US 5,145,684
Dissocubes®	SkyePharma	US 5,858,410
Nanopure	PharmaSol	PCT/EP00/0635
NANOEDGE™	Baxter	US 6,884,436

In the following, the different piston-gap homogenisation techniques are reviewed. Differences with regard to the physics are highlighted. Scaling up as a pre-requisite for the introduction of a product to the market and potential final formulations for the patient are discussed.

2.2.4 High pressure homogenisation (piston-gap)

2.2.4.1 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 5µm-25µm (varies with applied pressure and viscosity of the dispersion medium) when the suspension enters this homogenisation region (Figure 2-3).

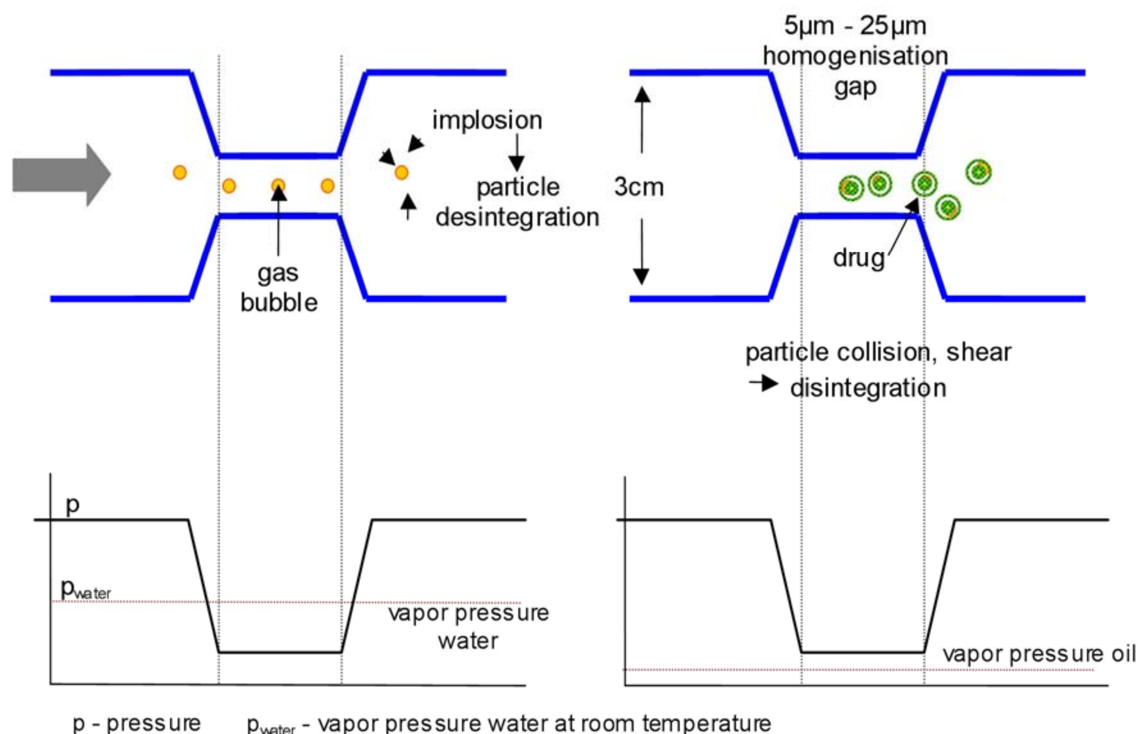


Figure 2-3: Change of the diameter of the streaming dispersion in a piston-gap homogeniser, from the cylinder containing the bulk suspension to the narrow homogenisation gap. The actual range of the static pressure as a function of the location inside the homogeniser is given in the diagrams below (left: situation for homogenisation in water, right: homogenisation in water-mixtures or water-free media).

According to the law by Bernoulli the flow volume of liquid in a closed system per cross section is constant. Thus, 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 to or 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, being again under normal air pressure conditions. For this first technology DissoCubes, the cavitation was considered as the determining factor outlined in the respective patent (Muller 1999).

As outlined in 2.2.3, it is important to have a pure drug nanocrystal suspension with low or very little presence of drug microparticles. To fully benefit from the decrease in particle size by diminution to nanocrystals, it is also important to have a mean diameter below 1µm. Independent on the actual drug nanocrystal size, at least the size should be homogeneous (as achieved with homogenisers) to avoid physical destabilisation by Ostwald ripening. The size of the drug nanocrystals that can be achieved mainly depends on the:

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 and the remaining crystals become more and more perfect. Thus, the force required to break the crystals increases with a 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. Hence, at this stage 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 the decrease in size and the 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 showed that above a certain pressure only a relatively small further decrease in size could be achieved (Fichera 2004). This can be explained, as the crystals are getting more and more perfect, whereas the force or energy required to break the crystals seems to increase rather exponentially. From this, for a drug particle with a given perfection in 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.

Figure 2-4 shows the decrease in the mean diameter (LD 50) of the bulk population of a nanosuspension as a function of homogenisation cycles (size measured by laser diffractometry (LD). LD yields a volume distribution that is expected to be 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 disappearance of large particles during the homogenisation process. Figure 2-4 shows that the maximum dispersity of the bulk population (LD 50) is already reached after 5 homogenisation cycles, afterwards 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) reach their maximum dispersity relatively fast. Further homogenisation cycles have little effect on the mean diameter of the bulk, but reduce the width of the distribution by eliminating remaining few large crystals in the second step. Therefore – even if 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 Oswald ripening and is also sensible when preparing formulations for i.v. injection to avoid capillary blockade.

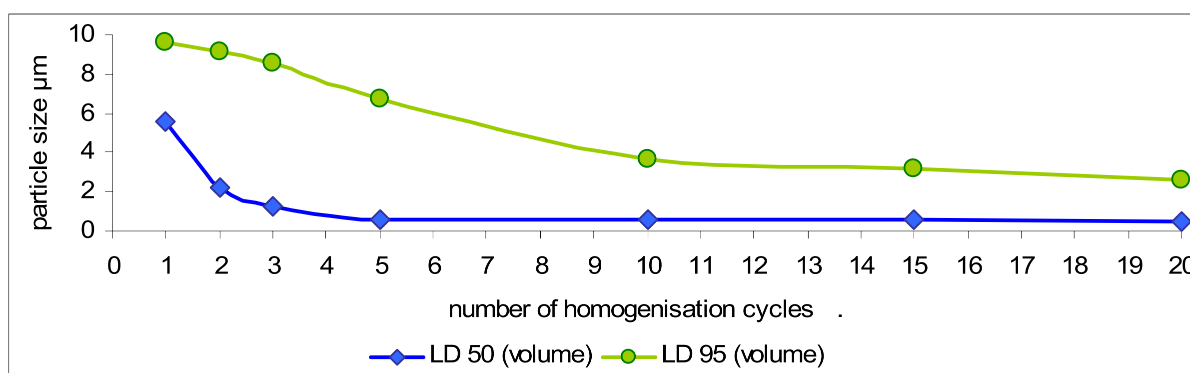


Figure 2-4: Azodicarbonamide (ADA): decrease in the mean diameter (LD 50) of the bulk population and reduction in the number of large particles, characterised by the LD diameter 95%, as a function of cycle numbers.

How it can 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 (Müller 1996) and coalesce. To some extent the same phenomena can occur in the homogenisation gap of a piston-gap homogeniser. According to the laminar 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 (Figure 2-5).

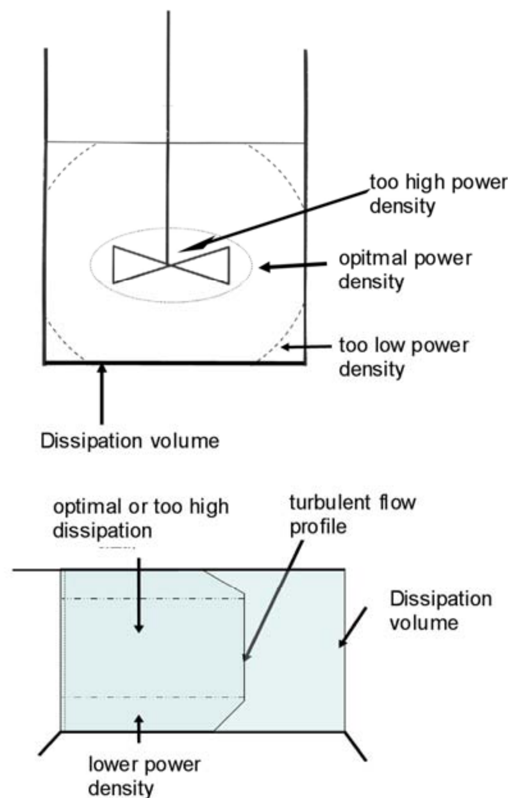


Figure 2-5: Power density distribution in a stirred beaker with zones of optimal (medium), too low and too high power density, leading to a polydisperse dispersion result (upper) and comparable situation in the gap of a piston-gap homogeniser with highest energy zone in the centre and lower streaming velocity close to the wall (lower).

With an increasing number of cycles, the probability increases that also these larger particles pass the zone of higher power density in the middle of the gap. Thus, finally also these particles are diminuted. Also the third effect discussed above for the process of emulsification of an oil, the coalescence in case of too high power density, can 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 to 10 homogenisation cycles, followed again by a decrease. This can be explained, as 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 250nm will be obtained at typical production conditions of 1500 bar and 10-20 homogenisation cycles (Böhm 1999). Diameters for other drugs reported are 493nm for amphotericine B, 519nm for itraconazole, 870nm for carbamazepine and 660nm for ketoconazole (Akkar 2004). A relatively hard drug processed by now is azodicarbonamide (ADA). Depending on the production conditions, a bulk population of around 800nm was found (Grau 2000) but by modification of the process also diameters of around 500-600nm could be obtained (Figure 2-4).

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 x interfacial tension ($\Delta E=\Delta H*\gamma$). 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, which 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 the opposite for drug crystals. These theoretical considerations are well in agreement with data reported in the literature. The maximum dispersity/smallest particle size of nanosuspensions does not depend on nature and concentration of stabilisers (Müller 1996). 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 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 RMKP98 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 of iron found in nanosuspensions was 0.7ppm, other metals present in the steel are present in much lower concentrations (Krause 2000).

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, which means within a suitable range.

2.2.4.2 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 (e.g. for HPMC capsules). 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 °C and 350 °C, respectively. As shown in Figure 2-3, 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 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 (Müller 1998). However, for chemically labile pharmaceutical compounds, homogenisation at around 80°C does not seem to be sensible (Müller 2000).

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 the 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) (Grau 2000). 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 amphotericine B in Figure 2-6. After 5 homogenisation cycles, the mean PCS diameter is around 600 nm and drops to about 200 nm after 25 cycles (Figure 2-6.).

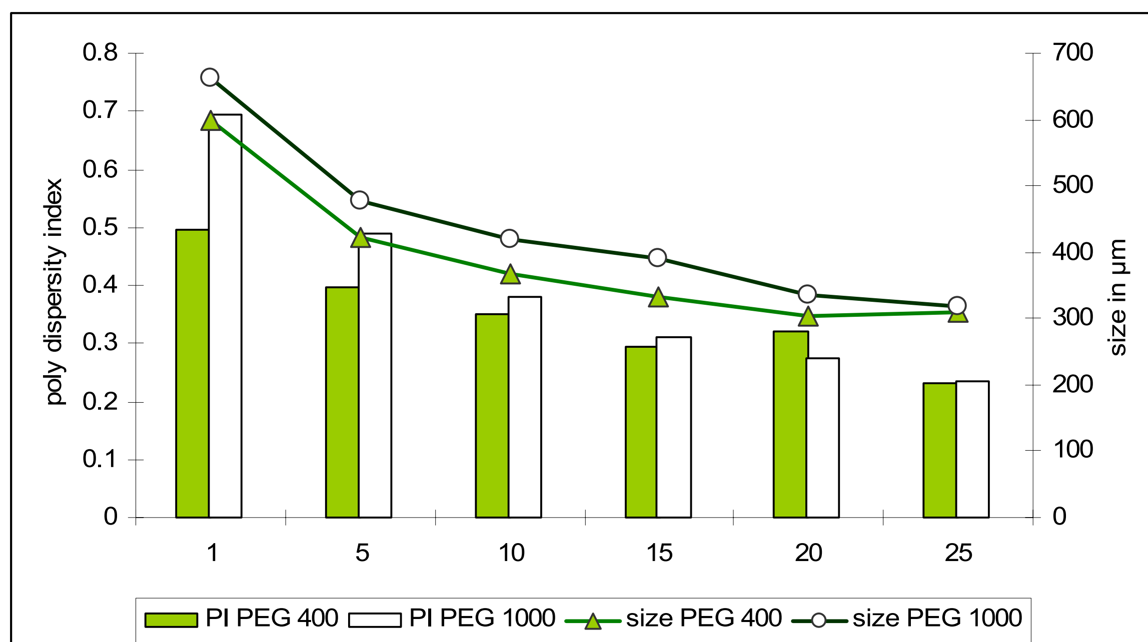


Figure 2-6: PCS diameter and polydispersity index (PI, measure of width of distribution) of amphotericine B powder homogenised in liquid PEG 400 and melted PEG 1000 (with permission after (Bushrab 2003)).

After homogenisation, the PEG drug nanosuspension is cooled, the PEG re-crystallises and fixes the drug nanocrystals inside the PEG matrix with a certain distance of the crystals to each other thus preventing crystal aggregation or crystal growth. For the production of the final dosage form, the liquid PEG nanosuspensions can be filled into capsules. Figure 2-7 shows the macroscopic appearance of liquid PEG nanosuspension, solidified PEG 1000 nanosuspension and the powder produced by grinding.

Figure 2-7: Amphothericin 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 (Bushrab 2003)).

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 with subsequent filling of the powder into hard gelatine or HPMC capsules (Bushrab 2003).

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

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. ethyl cellulose 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 50-400µm (Müller 2003). 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.

Amphotericin B drug nanocrystals were produced in glycerol-water mixtures (Buttle 2004). The optimum dispersity with avoidance of aggregation was found in a glycerol-water mixture of the ratio 80:20 (Buttle 2004). 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 amphotericine B emulsions for i.v. administration (so called SolEmuls technology (Müller 2001; Akkar 2003; Akkar and Muller 2003)). Because of their small size,

drug nanocrystals have excellent dissolution properties, thus allowing transferring amphotericine 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 amphotericine B products such as AmBisome (Schmidt 2002).

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, not covered by the DissoCubes® patent).

2.2.5 Combination technology precipitation and homogenisation (NANOEDGE)

As outlined above precipitated drug nanoparticles very often exhibit the tendency to continue crystal growth to the size of micrometer crystals. In addition, depending on the precipitation conditions the particles are completely amorphous, partially amorphous or crystalline. To ensure 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 during storage (shelf life of product) followed by a decrease in oral 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 (Kipp 2003). 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 ton. 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.

2.2.6 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, Niro Soavi, Lübeck, Germany). Increasing the produced volume from 3 ml to a batch size of about half a ton (500 kg) means an enlarging of 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 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 bar to 50 bar).

A modified LAB 60 homogenisation unit was built for the production of technical batches (Schneppe 2003). 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 litres. 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 airflow unit mounted at the ceiling of the production suite. 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 per hour at the maximum applicable pressure of 1500 bar. Alternatively, an Avestin 1000 could be used providing a homogenisation capacity of 1000 litres per hour. 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 ton nanosuspension would require 20 h (assuming 20 homogenisation cycles). In case of 4 homogenisers in series, this time would be reduced to 5 hours homogenisation time for 1000 kg of nanosuspension. Drug nanosuspensions can be produced with a 20% solid content without problems, in some cases nanosuspensions with a 40% solid content could be produced (Krause and Muller 2001). 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 in principle can also be applied for the Baxter combination technology NANOEDGE. For the production of sterile products, there are two possible approaches:

1. therminal sterilisation
2. aseptic production.

Therminal 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 (121ºC, 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 the 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 (e.g. thickness). 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 nanosuspensions on larger scale. Such a production line also has the advantage that highly potent drugs, such as cytotoxics, can be processed. In addition it should be born in mind that the 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.

2.2.7 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 (Charman 2000; Porter 2001; Porter and Charman 2001; Muller and Keck 2004). 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 and filled as powder in the capsules.

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 (Peters 1999). Alternatively, lyophilised products can be offered to be reconstituted prior to administration.

2.2.8 Products on the market/in clinical phases

Looking at the time between the invention of a technology and the first products on the market, this period is very short for the drug nanocrystals. The liposomes were invented in 1968 by Bangham and it took more than 20 years until the first pharmaceutical products appeared on the market at the beginning of the 90's. The first drug nanocrystal patents were filed at the beginning of the nineties 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 2mg of sirolismus. At the same time there is a solution on the market. The tablet has the advantage of being more user-friendly than the solution. Compared to the solution, the tablet has a 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 1-2mg, the total tablet weight is approximately 360mg. That means the drug nanocrystal content is below 1% causing no formulation difficulties.

The second product Emend was already introduced in the following year 2003 by the company Merck. It is a capsule containing 80 or 125mg of the drug aprepitant. The filling

materials of a capsule are pellets. A single dose of 125mg is in relation to typical weights of oral dosage forms between 400-500mg 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 90ties there was reluctance of pharmaceutical companies to employ the drug nanocrystal technology. At this stage it was a relatively new 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 pearl 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. Four products are on the market and quite a number is meanwhile in clinical phases. Table 2-3 gives an overview.

2.2.9 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 a bioavailability of 100% and allows pharmacological screening of any new chemical entity independent on its solubility properties. Other administration routes not discussed in this chapter are dermal delivery to create supersaturated systems with high thermodynamic activity, ophthalmic administration to create systems with prolonged retention times, nasal administration to adhere 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 tumors 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 (Müller 2001; Müller 2002; Müller 2003). 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 market and to the patient. Simple approaches can be realised much faster as proven by the first four nanocrystal products on the market since 2000. Moreover, a simple system does not mean necessarily that it is not smart. The smartness is the simplicity.

Table 2-3: Overview of drugs being presently marketed/ in different clinical phases, modified after (Rabinow 2004)

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	élan Nanosystems	Wyeth	oral	marketed
Emend	Anti-emetic	élan Nanosystems	Merck	oral	marketed
Tricor	lipid lowering	Abbott Laboratories	Abbott Laboratories	oral	marketed
Megace ES	weight gain	élan Nanosystems	Par Pharmaceuticalc	oral	marketed
cytokine inhibitor	Crohn's disease	élan Nanosystems	Cytokine PharmaSciences	oral	phase II
diagnostic agent	imaging agent	élan Nanosystems	Photogen	intravenous	phase I/II
thymectacin	anticancer	élan 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	élan 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

2.3 Particle size characterisation of nanosuspensions

The particle size characterisation of nanosuspensions is performed to obtain information about the main particle size of the systems and particle size distribution and about changes over time (e.g. crystal grows and agglomeration). As nanosuspensions consist of submicron particles, the appropriate method is photon correlation spectroscopy. PCS or dynamic light scattering analyses scattered laser light from particles diffusing in a low viscosity dispersion medium (e.g. water). PCS does not analyse the total intensity of the scattered light, basis of the analysis is the fluctuation velocity of the scattered light. The detected intensity signals (photons) are used to calculate the correlation function, from the decay of this correlation function the diffusion coefficient D of the particles is obtained. Applying the Stokes-Einstein equation, the mean particle size (called z-average) can be calculated. In addition a polydispersity index (PI) is obtained as a measure for the width of the distribution. The PI is 0 in case a monodisperse particle population is present. PI values of around 0.10 – 0.20 indicate a relatively narrow distribution, values of 0.5 and higher are obtained in case of very broad distributions. From the both obtained values (z-average and PI) even small increases of nanocrystal size with time can be detected. The extent of size increase is a measure for the extent of instability. Therefore PCS is a sensitive instrument to detect instabilities during long-term storage very early.

The measuring range of PCS is approximately 3nm-3µm. The size ranges for PCS given in the literature vary slightly, sometimes 5µm are stated as upper limit. The reason for these variations is that actually not the size itself but the density of the suspended particles is the limiting factor. PCS measures the diffusion velocity of the particles (not directly their size). Therefore, it is important that the measured particles undergo only diffusion caused by the Brownian motion of the dispersion medium molecules (e.g. water molecules). If there is a second velocity effect on the particles (e.g. sedimentation velocity), this velocity component would be also considered as diffusion velocity, that means sedimenting particles are virtually diffusing faster (superposition of diffusion velocity plus sedimentation velocity) and consequently the calculated PCS size would be much smaller. According to the definition drug nanosuspensions should have a mean size of the particles between a few nanometer and a maximum of 1000 nm. Therefore sedimentation effects are only of relevance if larger particles are present in the nanosuspension. Contamination with large crystals can be due to an inefficient diminution process (e.g. residual fraction of remaining microcrystals) or can occur during storage (aggregation, Ostwald ripening). Microcrystals larger 3µm will also affect the measured PCS particle size because they also scatter light as the small particles.

They have even a very distinct influence because their light scattering intensity is much higher than the one of small particles. In general the scattered light intensity (I) is proportional to the third/sixth power of the radius (depending on the particle size) (Xu 1997). Therefore when measuring a nanosuspension with a high content of microcrystals or crystal aggregates, the obtained PCS diameter does not reflect the mean size of the bulk population anymore as it is distinctly shifted to larger values. Therefore a complimentary sizing technology to PCS is required for the full characterisation of the drug nanocrystals. Laser diffractometry (LD) is mostly applied as the second characterisation method for nanosuspensions.

Laser diffractometry was developed around 1980 and found very fast its way as routine method in many laboratories of very different areas. The measurement range is depending on the manufacturer and ranges somewhere between 10nm and 8750µm. From this LD measurements are essential for full characterisation of drug nanocrystals, that means quantifying the percentage of microcrystals present, which is not possible by PCS measurements. LD analyses the Fraunhofer diffraction patterns generated by particles in a laser beam. The first instruments were based on the Fraunhofer theory which is – in a strict sense – only valid for particle sizes being 10 times larger than the wavelength of the light used for generating the diffraction pattern. For particle sizes below approximately 6.3 µm (in case of using a helium neon laser, wavelength 632.8 nm), the Mie theory needs to be applied to obtain correct particle sizes. The Mie theory requires the knowledge of the real refractive index of the particles and the imaginary refractive index (absorbance of the light by the particles). Unfortunately for most of the pharmaceutical solids the refractive indices is not known. However, laser diffractometry is frequently used as the second characterisation method for nanosuspensions, because of its often-called "simplicity". Nevertheless, laser diffractometry as a characterisation method was also reported to fail to characterise systems e.g. polydisperse emulsions correctly. Some publications are presently arguing against the meaningfulness of this technology (Driscoll et al. 2001; Burgess et al. 2004). The reliability of laser diffractometry in respect to its ability to detect larger particles within nanosuspensions was not investigated before. Detailed studies about the usefulness of this method for the characterisation of nanosuspensions and other nanoparticulate systems such as solid lipid nanoparticles (SLN, NLC) are not published until now. Doubts about the accuracy rise by the comparison of LD results obtained from various randomly selected nanosuspensions of different composition and age (Figure 2-8). Whereas data from LD 10-LD 90 vary tremendously, values for LD 100 seem to have either values e.g. 2.920, 3.206, 3.519, 4.241 or 6.158. Most of the suspensions analysed have an LD 100 of 4.656 (Figure 2-8).

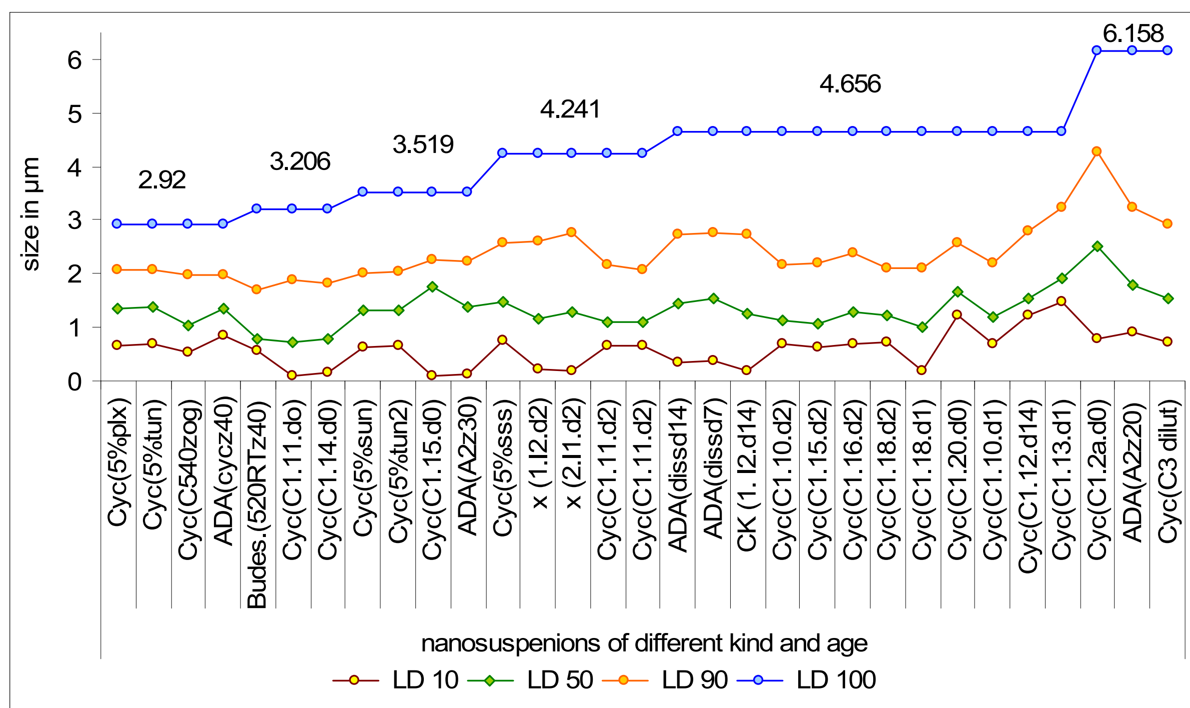


Figure 2-8: LD data for various different nanosuspensions, interestingly values of LD 100 from 42 different randomly selected suspensions had only six different values – indicating an error or poor resolution of the method?

Lager values for LD 100 are very seldom. The results are doubtful, especially in those cases, where macroscopic observation of the nanosuspensions already shows sedimentation effects of larger particles. Therefore, this method was subject to further and more detailed investigations within this thesis because of its great importance to characterise the nanosuspensions and to judge their physical long-term stability.