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Review

Nanocrystals: Industrially feasible multifunctional formulation technology for poorly soluble actives

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ABSTRACT

Poor solubility of new drugs and their related low oral bioavailability and general delivery problems are becoming a major challenge. Nanocrystals being a kind of "universal" formulation approach for these molecules are reviewed in this paper regarding the industrial feasibility, i.e. industrially available production processes (bottom–up and top–down technologies), regulatory aspects and nanotoxicology. This article also includes second generation nanocrystals («100 nm) as smartCrystals. The status of products on the market and in clinical phases is presented. The different special features of nanocrystals, which are exploited in different products, are described (tablets, capsule, aqueous nanosuspension). The main focus is given for oral and intravenous products. However, the potential and delivery strategies for other administration routes are discussed, i.e. dermal and mucosal, ocular, pulmonary and targeted delivery (e.g. via differential protein adsorption to the brain). In addition, the potential of the nanocrystal technology for delivery of poorly soluble, non-pharmaceutical actives is highlighted, i.e. in cosmetics or nutraceuticals.

Contents

1. Introduction

At present about 40% of drugs in the development pipelines and approximately 70% of drugs coming from synthesis or high throughput screening are poorly soluble in aqueous media, many as well simultaneously in organic solvents (Heimbach et al., 2007;

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Merisko-Liversidge and Liversidge, 2008). Poor solubility creates delivery problems such as low oral bioavailability and erratic absorption. Intravenous injection as an alternative route is not possible due to the large solvent volume required. Drug solubility can be enhanced using traditional approaches such as co-solvents, cyclodextrins or micronization. However, in many cases they cannot solve the bioavailability problem. For example, in case of many poorly soluble drugs micronization does not create a sufficiently large surface to adequately enhance the dissolution velocity. As a consequent next step one moved from micronization to nanonization, i.e. production of drug nanocrystals (Gao et al., 2008a; Müller et al., 2006; Rabinow, 2004).

The pharmaceutical benefits of nanocrystals include improvement in formulation performance, such as enhanced dissolution velocity and saturation solubility, reproducibility of oral absorption, improved dose-bioavailability, proportionality and increased patient compliance via reduction of number of oral units to be taken (Müller et al., 2001a; Rabinow, 2005). Nanocrystal serves as ideal delivery system for oral drugs having the dissolution velocity as rate limiting step for absorption, i.e. drugs of the biopharmaceutical classification system (BCS) class II and IV. In addition, nanocrystals can be injected intravenously as aqueous nanosuspensions (Rabinow et al., 2007). It is remarkable that how fast these nanocrystals entered the pharmaceutical market. It took about 25 years for the liposomes to appear in pharmaceutical products on the market (around 1990, e.g. Alveofact from Dr. Thomae GmbH (Diederichs and Müller, 1994). It was less than 10 years for the nanocrystals, having the first patent applications filed at the beginning of the 1990s (Müller et al., 1999), and the first product Emend® on the market in 2000. This short time confirms that it is an industrially feasible delivery system—in contrast to several "academic" developments. It is also developing as the most successful nanotechnology, when considering the block buster Tricor® (annual sales > 1 billion $ in US), and the number of products currently in clinical phases.

This article reviews briefly the production technologies in industry, the products on the market, and shows exemplarity, especially for nanocrystals exploited in the different oral products. Industrial nanocrystal development concentrates on oral products (élan, SkyePharma, Abbott), in second line on i.v. injectables (Baxter Healthcare). However, nanocrystals also possess great potential for use in other application routes, e.g. the dermal, ocular and pulmonary route including i.v. targeting. These opportunities are highlighted—considering the industrial requirements for product realization.

2. Industrial production of nanocrystals

Two basic approaches are involved in production of nanocrystals, the bottom–up technologies (controlled precipitation/crystallization) and the top–down technologies, nanonizing (large-size drug powder to be reduced in size, e.g. by mechanical attrition). However, the combination techniques, combining a pre-treatment with a subsequent size reduction step are also being employed. A recent review focuses on the various production technologies available, also e.g. solvent-evaporation and supercritical fluid technologies (Arunkumar et al., 2009), which are not covered in this review because they are less industrially relevant at present.

2.1. Bottom–up—precipitation methods

Historically, around 1980 Sucker developed the so called "hydrosols", the intellectual property acquired by Sandoz (nowadays Novartis) (List and Sucker, 1988; Suker and Gassmann, 1994). This technology is basically a classical precipitation process known as "via humida paratum" (VHP), where drug is dissolved in a solvent and subsequently precipitated by mixing with a non-solvent. It yields crystalline drug nanoparticles. This method requires strict control of the process, avoidance of crystal growth (to the micrometer range), drug solubility in at least one solvent, and of course has the problem of solvent residues. Due to the complexity of process, as per our knowledge there are no pharmaceutical products on the market based on this technology.

Another precipitation process was developed by Auweter and Horn (Auweter et al., 1998), leading to amorphous nanoparticles of the active. The particles are spherical due to precipitation process (Fig. 1, left). This process is used by BASF for products developed in the food sector (e.g. Lucarotin® or Lucantin® which is a solution of the carotenoid, together with a surfactant in a digestible oil), and for pharmaceuticals by Soliqs® (Abbott GmbH & Co. KG, Ludwigshafen) previously Knoll/BASF. The Soliqs trade name is NanoMorph®. Theoretically, a particle being in the nano range and at the same time amorphous is ideal (Auweter et al., 2002); it has the highest increase in saturation solubility. However, there is a risk that the amorphous active can re-crystallize; in this case pharmaceutical product leads to a decrease in the oral bioavailability. Partial re-crystallization is less or not critical in food products, therefore by now products in this sector are on the market. Whereas in pharmaceuticals, the formulation technology is available, but not yet introduced into products. After introduction of crystalline nanoparticles to the market, amorphous nanoparticles might belong to the second improved generation, because of their superior dissolution velocity and higher solubility. Another bottom–up process is controlled crystallization during freeze drying (de Waard et al., 2008), which is also considered to be suitable for large-scale production (de Waard et al., 2009).

2.2. Top–down technologies

2.2.1. Bead/pearl milling

The NanoCrystals® technology by élan (prev. Nanosystems) uses a bead/pearl mill to achieve particle size diminution. It was developed by Liversidge et al. (Liversidge et al., 1992). Milling media, dispersion medium (generally water) containing stabilizer along with drug are charged into a milling chamber. Shear forces generated by the movement of the milling media lead to particle size reduction. Smaller or larger coated milling pearls of ceramics (cerium or yttrium stabilized zirconium dioxide), stainless steel, glass or highly crosslinked polystyrene resin-coated beads can be used. Erosion from the milling material during the milling process is a common problem of this technology. The milling time mainly depends on the hardness of the drug, viscosity, temperature, energy input, size of the milling media and surfactant concentration used. The milling time can last from about 30 min to hours or several days. This is an important industrially used technology for particle size reduction, proven by the FDA-approved products (cf. also Table 2).

Typically lab scale production can be carried out at using 100 mg or less of API by using the Nanomill® system (élan Drug Discovery, PA, USA). The chemical form of API needs to be considered for laboratory testing, typically the neutral form is preferred. Production volumes of more than 5 L (flow through mode) can be produced using the Dynomill (Glen Mills, Inc. Cliffton, NJ, USA) with chamber size of 300 and 600 mL. Also larger sized mills are available (e.g. Netzsch mills (Netzsch Inc., Exton, PA, USA)), e.g. in 2, 10 and 60 L chamber size. Scaling up with a pearl mill is possible, but there is a certain limitation in size of the mill due to its weight. To produce larger batches the mills can be configured in the circulation mode. The NanoCrystal® technology has successfully expanded the use of nanosuspensions for oral, inhalation, intravenous, subcutaneous, intramuscular and ocular delivery (Merisko-Liversidge and Liversidge, 2008).

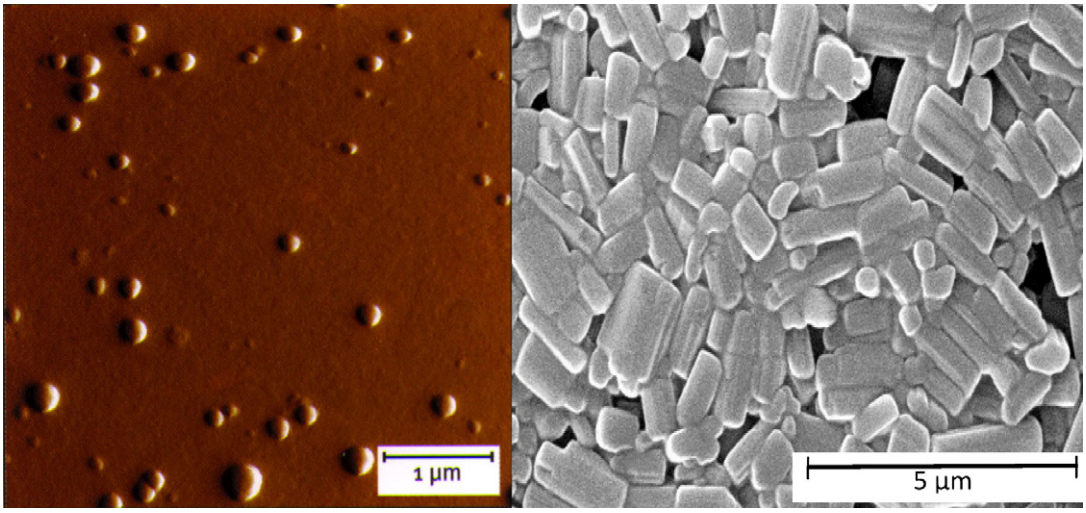

Fig. 1. Nanomorph® drug nanoparticles (left)—they are spherical because of the amorphous state (by courtesy of Soliqs/Ludwigshafen), and cuboid formed crystalline nanoparticles (nanocrystals, right) (modified after Böhm, 1999).

2.2.2. High pressure homogenization

The three basic processes used are Microfluidizer technology (IDD-P™ technology) based on the jet stream principle (Haynes, 1992), piston-gap homogenization either in water (Dissocubes® technology, SkyePharma) (Müller et al., 1992) or alternatively in water-reduced/non-aqueous media (Nanopure® technology, prev. PharmaSol GmbH, Berlin, now Abbott Laboratories) (Müller et al., 2001b).

The Microfluidizer technology is based on the jet stream principle and can generate small particles by a frontal collision of two fluid streams in a Y-type or Z-type chamber under pressures up to 1700 bar. The jet streams lead to particle collision, shear forces and cavitation forces (Microfluidizer®, Microfluidics Inc.). Often a relatively high number of cycles (50–100 passes) are necessary to obtain sufficient particle size reduction. SkyePharma Canada Inc. (formerly Canadian company Research Triangle pharmaceuticals (RTP)) uses this principle for their Insoluble Drug Delivery-Particles (IDD-P™) technology to achieve the production of submicron particles of poorly soluble drugs (Keck and Müller, 2006). Product on the market is Triglide®, fenofibrate (cf. Table 2).

The Dissocubes® technology was developed by Müller and co-workers by employing piston-gap homogenizers (e.g. APV Gaulin/Rannie homogenizers). The technology was acquired by SkyePharma PLC in 1999. A drug dispersed in an aqueous surfactant solution (macrosuspension) is forced by a piston under pressure (up to 4000 bar, typically 1500–2000 bar) through a tiny gap (e.g. 5–20 μm). The resulting high streaming velocity of the suspension causes an increase in the dynamic pressure. This is compensated by a reduction in the static pressure below the vapor pressure of the aqueous phase; hence, water starts boiling forming gas bubbles. These gas bubbles collapse immediately when the liquid leaves the homogenization gap (=cavitation). The drug particles are reduced in size due the high power of the shockwaves caused by cavitation. The mean size of bulk population obtained for the high pressure homogenization process depends on the power density of the homogenizer (homogenizer pressure), number of homogenization cycles and hardness of drug (Müller et al., 2000). Because of crystalline nature they appeared in cuboid or irregular shape (Fig. 1, right), in contrast to spherical amorphous drug nanoparticles.

The Nanopure® technology is another approach using the piston-gap homogenizer (prev. PharmaSol GmbH, now Abbott). This technology uses a primary dispersion medium, non-aqueous liquids, e.g. oils, liquid and solid (melted) PEG, or water reduced media (e.g. glycerol–water, ethanol–water mixtures), and optionally homogenization at low temperatures. These media have low vapor pressure, cavitation takes place very limited or not at all. At homogenization at room temperature, the water starts boiling, i.e. the static pressure on the water is reduced to the vapor pressure of water at 20 °C, being 23.4 (weblink1). For example, the vapor pressure of Miglyol 812 oil is only 0.01 hPa (=0.01 mbar) at 20 °C (weblink2), i.e. more than 2000 fold lower. Therefore when water shows cavitation, the oil will not. Even without cavitation, the size diminution is sufficient because of shear forces, particle collisions and turbulences. The optional low temperatures allow the processing of temperature sensitive drugs, in addition at lower temperatures materials, are more fragile. Final nanosuspensions product in oil or PEG can be directly filled into gelatin or HPMC capsules.

2.2.3. Combination technologies

These technologies combine a pre-treatment step with a subsequent high energy step, for example – but not necessarily – high pressure homogenization. The NANOEDGE™ technology by Baxter uses a first classical precipitation step with a subsequent annealing step by applying high energy, e.g. high pressure homogenization (Kipp et al., 2001). According to the patent claims, the annealing step prevents the growth of the precipitated nanocrystals. Annealing is defined in this invention as the process of converting matter that is thermodynamically unstable into a more stable form by single or repeated application of energy (direct heat or mechanical stress), followed by thermal relaxation. This lowering of energy may be achieved by conversion of the solid form from a less ordered to a more ordered lattice structure. Alternatively, this stabilization may occur by a reordering of the surfactant molecules at the solid–liquid interface.

A problem is the use of organic solvents in the precipitation step. In case of large-scale production relatively large amounts of solvent need to be removed, and removal needs to take place in a sterile production process—making it even more tricky and expensive. The Baxter developments focus mainly on i.v. injectables. Due to potential stability impairment of aqueous nanosuspensions by terminal sterilization (autoclaving, irradiation), in general production has to take place under aseptic conditions, including the homogenization line.

The smartCrystal® technology is owned by Abbott and marketed by its drug delivery company Soliqs in Ludwigshafen/Germany. It is a family of various combination processes, a kind of tool box to tailor-make the nanocrystals for each specific application, and

Table 1
Combination processes of the smartCrystal technology, combining a pre-treatment step with subsequent high pressure homogenization (HPH), apart from Nanopure, which is using modified dispersion media (e.g. oil, polyethylene glycols, water–glycerol).

Process code	Pre-treatment	Main treatment	Patent no. and date of filing
Nanopure	No pre-treatment, dispersion media: non-aqueous or water mixtures	HPH	PCT/EP00/06535, 2000
H 42	Spray-drying	HPH	DE/102005 011 786.4, 2005
H 69	Precipitation	HPH	PCT/EP 2006/009930, 2007
H 96	Lyophilization	HPH	PCT/EP 2006/003377, 2007
CT	Pearl/bead milling	HPH	PCT/EP 2007/009943, 2006

considering the physical properties of the drug (e.g. hardness). It was acquired from PharmaSol GmbH in 2007, and these crystalline nanoparticles complement to the amorphous nanoparticles by Soliqs, NanoMorph® (cf. 2.1) (Fig. 1). Nanopure® process listed under the smartCrystal family, Table 1 gives an overview. Special feature of the processes H69 and H96 is the ability to produce crystals below 100 nm, a range practically not accessible by high pressure homogenization alone. Spray-drying or lyophilization of the drug solution leads to a powder more susceptible to be broken in the subsequent high pressure homogenization step. The smartCrystal technology is considered as the second generation of drug nanocrystals (Keck et al., 2008). Injecting nanosuspensions with very small nanocrystals can permit fast dissolution and mimic injection of a solution.

3. Status of nanocrystals in the market

Nanocrystals for oral administration were the first products on the pharmaceutical market – due to the huge market potential – and the easier way of product realization compared to the intravenous route. Various products exploited use different features of the nanocrystals, an overview of market products is given in Table 2.

The first nanocrystal product Rapamune® (rapamycin, immunosuppressive) was placed on market in year 2000 by Wyeth. The tablet contains 1–2 mg sirolimus, the tablet weight is about 370 mg. The low loading with nanocrystals excluded problems during tablet compression (e.g. nanocrystals aggregation). To achieve a sufficiently high bioavailability (BA) for sirolimus one needed a solution. This was not convenient for the patient. The drug nanocrystals tablet is convenient and performs even better than the solution. The BA of the tablet is 21% higher than the BA of the solution (i.e. solution = 100%, tablet = 121%). A possible explanation is, that the drug solubility is higher when dissolving from nanocrystals (kinetic saturation solubility > saturation solubility of solution), the increased concentration gradient increases absorption.

In 2001, the second product Emend® was introduced by the company Merck (aprepitant capsule, antiemetic). The single dose of 80 and 125 mg, and incorporated into pellets, filled in a hard gelatin capsule. The nanocrystal loading is much higher compared to Rapamune. Therefore making pellets by extrusion as a lower energy process instead of compression minimized risk of nanocrystals aggregation. In addition, pellets can be easily divided in smaller doses. The nanocrystal feature exploited is the fast dissolution, because aprepitant has an absorption window in the upper GI tract.

The next product was Tricor® (fenofibrate tablet for hypercholesterolemia, Abbott Laboratories). Another product with fenofibrate nanocrystals is Triglide (hypercholesterolemia) which is produced by SkyePharma based on the IDD-P® technology and marketed by Sciele Pharma Inc. (Atlanta, CA, USA). Tricor® has a dose of 48 or 145 mg and given as a tablet form. Tricor is the successor product for fenofibrate after patent expiry. The nanocrystals technology served for life-time extension, and at the same time led to a superior product performance. Fenofibrate showed 35% higher absorption in the fed state. The nanocrystals significantly reduced the differences between non-fed/fed status. This is due to their adhesive properties of nanocrystals which are not much affected by the nutritional state of patient.

Par Pharmaceutical Companies Inc. (Spring Valley, NY, USA) introduced Megace ES® (ES for enhanced solubility) for the delivery of megestrol acetate (a synthetic progestin, antianorexic). The name was licensed from Bristol Myers Squibb (New York). The interesting feature is that it is not a solid oral dosage form, but an aqueous nanosuspension. The dose is 625 mg/5 mL. The nanosuspension also reduces the differences in BA for non-fed/fed condition similar as in Tricor. In addition, it has less administration volume than previously given oral formulation (only 1/4) and is less viscous. Nanosuspensions are ultrafine systems with a high surface energy, and are often considered being of critical physical stability (e.g. Ostwald ripening). The product Megace ES® proves that aqueous nanosuspensions can be produced with sufficient physical stability for the shelf life of a product. Other products on the market and clinical trials are listed in Tables 2 and 3, respectively.

4. Application routes—exploitable principles of nanocrystals action

4.1. Oral drug delivery

The biological activity/oral BA of a compound depends on its ability to dissolve and diffuse through the gastrointestinal membranes to the blood. In BCS class II the BA is limited by the dissolution velocity, in class IV there is additionally a transport mechanism reducing the absorption (e.g. p-glycoprotein). In the latter case, absorption might be enhanced by flooding the trans-

Table 2
Examples of nanocrystal products on the market.

Trade name	Therapeutic use	Applied technology	Pharma company	Administration route
Rapamune® (Rapamycin, Sirolimus)	Immunosuppressive	*élan* nanosystems	Wyeth Pharmaceuticals	Oral
Emend® (Aprepitant)	Antiemetic	*élan* nanosystems	Merck & Co.	Oral
Tricor® (Fenofibrate)	Hypercholesterolemia	*élan* nanosystems	Abbott Laboratories	Oral
Triglide® (Fenofibrate)	Hypercholesterolemia	IDD-P® technology	Produced by SkyePharma marketed by Sciele Pharma Inc. (Atlanta, CA, USA).	Oral
Megace ES® (Megestrol acetate)	Antianorexic	*élan* nanosystems	Par Pharmaceutical Companies Inc. (Spring Valley, NY, USA)	Oral
Avinza® (Morphine sulfate)	Psychostimulant drug	*élan* nanosystems	King Pharmaceuticals	Oral
Focalin® XR (Dexmethyl-phenidate HCl)		*élan* nanosystems	Novartis	Oral
Ritalin® LA (Methylphenidate HCl)		*élan* nanosystems	Novartis	Oral
Zanaflex Capsules™ (Tizanidine HCl)	Muscle relaxant	*élan* nanosystems	Acorda	Oral

Table 3
Overview of various drug candidates in clinical trials.

Trade name	Therapeutic use	Applied technology	Pharma company	Administration route	Status (Phase)
Fenofibrate	Lipid lowering	SkyePharma	Undisclosed	Oral	I
Insulin	Diabetes	BioSante	Self developed	Oral	I
Busulfan	Anti-cancer	SkyePharma	Supergen	Intrathecal	I
Budesonide	Asthama	*élan* Nanocrystal	Sheffield Pharmaceuticals	Pulmonary	I
Calcium phosphate	Mucosal vaccine adjuvant for herpes	Biosante	Self developed	Oral	I
Thymectacin	Anticancer	*élan* Nanocrystal	NewBiotics/IIex oncology	Intravenous	I/II
Megesstol Acetate	AIDS related weight loss	*élan* Nanocrystal	Par Pharmaceuticals Inc.	Oral	II
Panzem® NCD (2-methoxy estradiol)	Ovarian cancer	*élan* Nanocrystal	EntreMed	Oral	II
Panzem® NCD	Recurrant glioblatoma multiforme	*élan* Nanocrystal	EntreMed	Orally	II
Panzem® NCD and Tamozolomide	Anti-cancer	*élan* Nanocrystal	EntreMed	Oral	II
Panzem® NCD and Avastin (Bevacizumab)	Carcinoid tumor	*élan* Nanocrystal	EntreMed	Panzem-Orally Bevacizumab-Intravenously	II
Panzem® NCD with and without Sanitinib Malate	Renal cell carcinoma	*élan* Nanocrystal	EntreMed	Oral	II
Panzem® NCD	Prostate cancer	*élan* Nanocrystal	EntreMed	Oral	II
Fenofibrate	Sleep apnea syndrome	*élan* Nanocrystal	Solvay Pharmaceuticals	Oral	II
Undisclosed	Antiinfective	Baxter NANOEDGE	undisclosed	Oral/Intravenous	II
Cytokine inhibitor	Crohn's disease	*élan* Nanocrystal	Cytokine Pharmasciences	Oral	II
Guanylhydrazone (Semapimod®)	TNF-alpha inhibitor	Self developed	Cytokine Pharmasciences	Intravenous	II
Themectacin (Theralux™)	Anticancer	*élan* Nanocrystal	Celmed	Intravenous	II
Silver (Nucryst®)	Atopic dermatitis	Self developed	Nucryst Pharmaceuticals	Topical	II
Paclitaxel (Paxceed™)	Anti-inflammatory	Unknown	Angiotech	Intravenous	III
Paclitaxel	Anticancer	Unknown	American Pharmaceutical Partners	Intravenous	III

porter system with dissolved drug. The faster dissolution of the nanocrystals can be explained by the increase in surface area (*A*) when moving from micronized to nanonized particles (Fig. 2, lower). At the same time, the saturation solubility Cs increases below a size of about 1 μm. The basis for this is the Kelvin equation describing the vapor pressure of a liquid droplet in a gas phase, which corresponds to the dissolution pressure of a solid particle in a liquid. The Cs depends on the size, i.e. the curvature of the particle and the corresponding dissolution pressure. The dissolution pressure increases with increasing curvature, i.e. decreasing particle size (Fig. 2, upper). The vapor/dissolution pressure can be

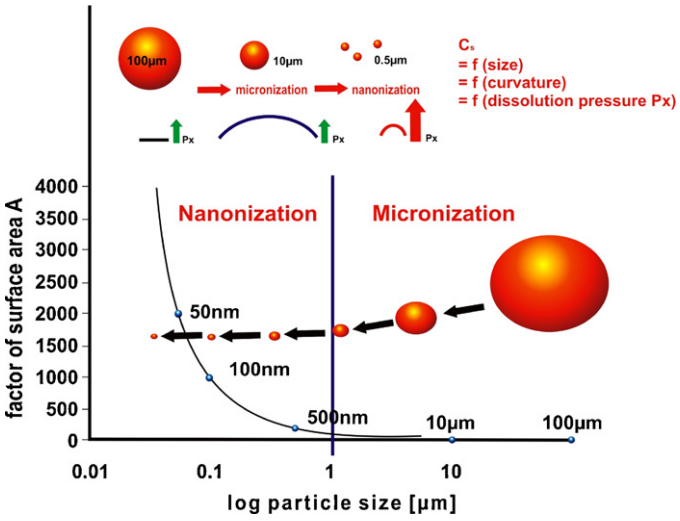

Fig. 2. Change in properties when moving from micronization to nanonization. Upper: the size reduces, the curvature increase accompanied by an increase in dissolution pressure p_x, i.e. increase in saturation solubility. Lower: size reduction leads to an increase in surface area, being pronounced below about 1 mm, and very pronounced below 100 nm, the very small nanocrystals of the second generation (by courtesy after Mauludin, 2008).

calculated as a function of size, showing a steep increase below 1 μm, a very pronounced increase below 100 nm (Müller and Akkar, 2004). The increased dissolution pressure shifts the balance of dissolving/re-crystallizing molecules around a crystal towards the dissolved molecules. A higher kinetic saturation solubility than the thermodynamic equilibration solubility leads to an increased concentration gradient at membranes, subsequently leading to higher penetration or permeation. Both increased Cs and increased surface area enhances the dissolution velocity, as described in the equation by Noyes–Whitney (Noyes and Whitney, 1897). By now, main attention was focussed on size and related surface area. It was recently reported that the interfacial reaction resistance is getting the velocity determining parameter for crystals below 1 μm. Design of nanocrystals with faster interfacial reaction can further enhance the dissolution velocity (Crisp et al., 2007). In addition, nanomaterials possess improved adhesiveness to biological membranes.

In many studies, these principles of actions for nanocrystals were employed. Administration of atovaquone (an antibiotic used in treating opportunistic Pneumocystis carinii infections) as a nanosuspension resulted in a 2.5-fold increase in oral bioavailability as compared to the commercial product Wellvone, which contains the micronized drug (Schöler et al., 2001). The enhancement in oral bioavailability can be attributed to the adhesiveness of the drug nanosuspension, increased surface area (due to reduction in particle size by 10 to 50 fold) and saturation solubility. Danazol (gonadotropin inhibitor) showed a drastic improvement in bioavailability to 82.3% when administered as a nanosuspension as compared to marketed danazol macrosuspension (Danocrine) which showed poor bioavailability of 5.2% (Liversidge and Cundy, 1995). Oral administration of amphotericin B as a nanosuspension produced a substantial improvement in its oral absorption in comparison to orally administered conventional commercial formulations such as Fungizone, AmBisome and micrometer amphotericin B (Kayser et al., 2003). Apart from improving oral absorption, nanosuspensions offer improved dose proportionality, reduced fed/fasted state variability and reduced inter-subject variability. Another examples for oral nanocrystals is the anthelmintic

drug albendazole, showing an about five times higher saturation solubility compared to the raw material (Ravichandran, 2010). In another study 1,3-Dicyclohexylurea (DCU) was formulated as a nanosuspension to lower systemic blood pressure. The test model was hypertensive rats dosed intraperitoneally. The DCU nanosuspension administered orally twice daily yielded plasma exposures an order of magnitude greater than the raw material (Ghosh et al., 2008).

Nanosuspensions exhibit a quick onset of action for drugs that are completely but slowly absorbed like naproxen (NSAID) for which dosage form with fast onset of action would be highly desirable. Naproxen nanosuspension showed a reduction in t_{max} by approximately 50% to achieve C_{max} as compared to the suspension (Naprosyn) and the tablet (Anaprox), besides an increase in bioavailability (Merisko-Liversidge et al., 2003). In some cases, quick onset and the associated high C_{max} is not desired. In this case, the nanocrystals need to be incorporated into prolonged release dosage forms, e.g. pellets (Möschwitzer and Müller, 2006). This is sensible approach for delivery of BCS class IV drugs, addition of inhibitors of the efflux transporters.

Nanocrystals can be easily transformed to solid oral dosage forms. The aqueous nanosuspension can be used as granulation fluid in tablet production, or as wetting fluid for the mass in pelletization. Aqueous nanosuspensions can be transferred to powders by spray-drying or lyophilization. Important is that the dry product redisperses well in water, with little increase in the size (i.e. little aggregates) (Fig. 3). The powder can be compressed into tablets. The enhanced dissolution behaviour for a rutin tablet made from nanocrystals compared to a marketed product is shown in Fig. 4. Even larger differences to marketed products were obtained for the nutraceuticals coenzyme Q10 and hesperidin (Mauludin, 2008).

Fig. 3. Aqueous coenzyme Q10 nanosuspensions, size 219 nm (left), lyophilized nanocrystal power (middle) and redispersed lyophilized powder in Milli-Q water (right). The size after redispersion is only slightly higher, 263 nm (photon correlation spectroscopy data) (by courtesy after Mauludin, 2008).

4.2. Parenteral/intravenous administration

Parenteral administration of poorly soluble drugs requires often the use of a solubilization technology, at least when doses have to be administered being not soluble in typical injection (1–10 mL) or infusion volumes (e.g. 100 mL). Approaches being used are based on solubilization by surfactants (e.g. Cremophor® EL in Taxol®), solvent mixtures (e.g. ethanol–water) or cyclodextrins inclusion complexes. However, many drugs of today are so poorly soluble that these approaches do not work. Or these approaches work, but are associated with undesired side effects. Examples are anaphylactic reaction in products with Cremophor® EL, (Irizarry et al., 2009) or nephrotoxicity when used cyclodextrins intravenously (Rabinow et al., 2007). Nanodelivery systems are of increasing interest for not only oral but also parenteral administration, e.g. microemulsions but also nanosuspensions as described for benzimidazoles (Chow et al., 2010). Nanosuspensions are a smart approach to solve both problems i.e. insufficient solubility and side effects. They can be produced using well-tolerated stabilizers, and can be injected in concentrations up to 10% (w/w) without obvious problems (unpublished data from our animal studies).

The product Sporanox® IV (itraconazole, Janssen Pharmaceutical Products, L.P.) exhibited significant acute toxicity above 10 mg/kg and an LD50 value lower than 40 mg/kg when administered as a bolus in the caudal vein of rats. Itraconazole nanosuspension could be administered up to 320 mg/kg without animal mortality. Itraconazole nanosuspensions were developed and intensively investigated by Baxter Healthcare (Pandey, 2010; Rabinow et al., 2007). The maximum tolerable dose of paclitaxel nanosuspension was found to be three times higher than the currently marketed Taxol (Böhm, 1999). To optimize the stability of nanocrystal suspensions can be a rather complex process as shown for specifically paclitaxel (Deng et al., 2010). There is a complex

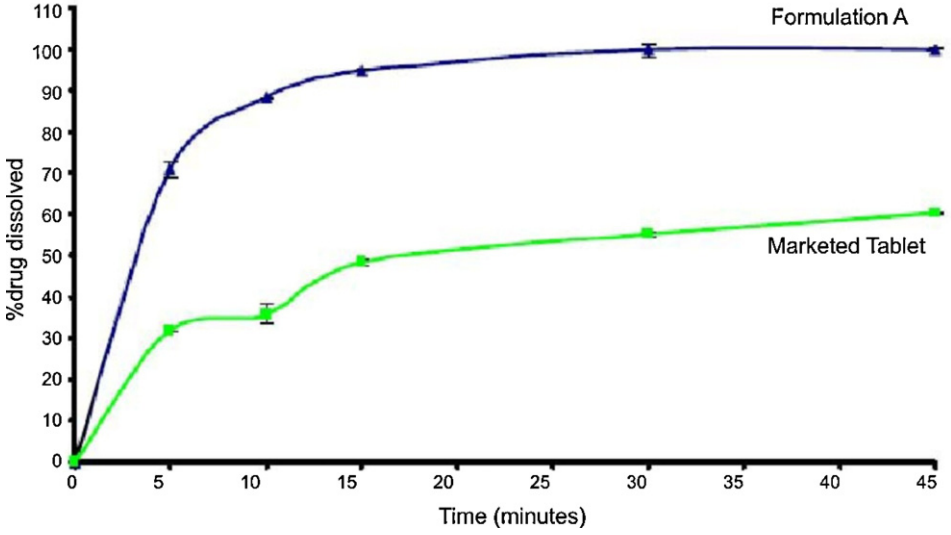

Fig. 4. Percentage of dissolved rutin from nanocrystal formulation A versus a marketed tablet made containing rutinosid (non-nanonized) with a content of 50 mg rutin,(by courtesy after Mauludin, 2008).

process of adsorption and desorption of polymeric stabilizer on the nanocrystals surface, depending on polymer concentration and temperature. Similarly to paclitaxel nanosuspension, the nanosuspensions of etoposide and camptothecin revealed an improvement in the tolerance level of the drug compared to the marketed preparations (Merisko-Liversidge et al., 2003). Clofazimine (antileprotic drug) nanosuspension showed an improvement in stability and efficacy over the liposomal clofazimine (Peters et al., 2000). A nimodipine i.v. nanosuspension was developed to have an injectable form better tolerated than the commercially available ethanol solution. Irritability studies in rats showed less local irritation and less phlebitis risks (Xiong et al., 2008).

It has to be taken into account, that i.v. injected nanocrystals exhibit a different pharmacokinetics compared to an injected solution, when the nanocrystals are larger than 100 nm. The nanocrystals do not dissolve fast enough and are sequestered by the mononuclear phagocytic system (MPS) cells. They accumulate mainly in the Kupffer cells of the liver like other colloidal drug carriers, e.g. shown for Paclitaxel nanosuspensions (Böhm, 1999). That means a generic product to Taxol® cannot be produced using such large-sized nanocrystals. As positive effect, this accumulation in the liver leads to prolonged plasma levels. The macrophages act as depot and drug is being released over time to the blood. As potential side effect, local high drug concentrations can cause toxicity to liver. One needs to find a balance by selecting the right injected dose. Asulacrine nanosuspension was developed for i.v. treatment of breast cancer. It showed a different pharmacokinetics to the injected solution, and enrichment in liver, lung and kidney (Ganta et al., 2009).

Injected nanocrystals can also be used to target drugs to various vital organs (Shegokar, 2010). Accumulation in the liver is "natural" or "passive" targeting, it happens automatically. To achieve macrophage targeting in certain diseases like HIV/AIDS, tuberculosis (TB) the surface properties of nanosuspensions could be modulated in a controlled way to alter the plasma protein adsorption pattern. This directs particles away from the liver to e.g. the spleen. The uptake pattern within the different cells of the MPS can be changed to treat most efficiently a disease.

To direct nanocrystals to other sides in the body (=active targeting), identical to stealth liposomes, the surface of the nanocrystals needs to be masked to avoid opsonin adsorption and recognition by the MPS (Shegokar and Singh, 2009). In addition, the surface coating needs to have attached a targeting moiety, e.g. antibody or targeting protein (Shegokar et al., 2010c). Kreuter et al. were successful to target darlagin loaded onto polymeric nanoparticles to the brain. The particles were surface-modified by Tween 80 adsorption (Kreuter et al., 1997). This coating leads to preferential adsorption of Apolipoprotein E after i.v. injection. The Apo E mediated the targeting to the endothelial cells of the blood–brain barrier (Lück, 1997). This principle was transferred to atovaquone nanocrystals. The parasites in toxoplasmosis could be eradicated in the brain (Schöler et al., 2001). The challenge in targeting to other sides than the liver and spleen is to enrich a sufficiently high percentage of the injected dose in the target area. There is still a competition in uptake by the MPS and the target area. In brain delivery, it is estimated that not more than about 1% of the injected dose reaches the brain. This is the present hurdle for targeting with i.v. nanocrystals.

To mimic injected solutions, the nanocrystals need to dissolve very fast, i.e. they should be «100 nm. The difference in organ distribution as a function of nanocrystals size was shown for oridonin nanosuspensions with a size of 103 nm versus 897 nm. The small nanocrystals exhibited a pharmacokinetics and organ distribution similar to the injected solution, the large nanocrystals accumulated in the RES organs (Gao et al., 2008b). Of course, it does not depend on the size only, but also on the compound specific dissolution pressure. For such formulations, the second generation nanocrystal technology is required providing such small sizes. Moreover, nanosuspensions can also be administered via other parenteral routes, e.g. intraarticular and intraperitoneal. Replacing an intraperitoneally injected microsuspension by a nanosuspension could avoid irritation of the peritoneum (unpublished data). Wolf et al., have published preclinical studies for subcutaneous application of nanocrystal formulations (Wolf et al., 1999). However, by now these routes are not intensively studied, almost nothing is published.

Nanocrystals are also a formulation principle in early drug discovery and screening. In the assessment of pharmacodynamic responses in early drug screening, very often one needs "tool compounds". These tool compounds are often poorly soluble. The poorly soluble 1,3-Dicyclohexylurea (DCU) reduces blood pressure, and was formulated as nanosuspension for intravenous bolus and infusion dosing (Wahlstrom et al., 2007).

4.3. Dermal and mucosal application

Nanocrystals exhibit the properties like increased penetration into a membrane, enhanced permeation and bioadhesiveness. These principles were exploited to the gastrointestinal wall for the oral products. The injectability and fast dissolution was exploited for intravenous formulation developments. However, for many years no attention was given to exploit adhesion, fast dissolution and increased penetration for dermal and mucosal application.

This changed when the poorly soluble antioxidants rutin, apigenin and hesperidin were formulated as nanosuspension for application in skin-protective, anti-aging cosmetic products (Al Shaal et al., 2010a; Mauludin et al., 2009). The nanocrystals are simply admixed to the water phase of dermal creams and o/w lotions. The first products with rutin appeared on the market in March 2007, series Juvedical, age-decoder face cream and fluid. It was followed by Platinum Rare in 2009, containing hesperidin. These products contain nanosized crystals, but are not a nano product according to the new European regulations for cosmetics (weblink3), as the size of the nanocrystals is above 100 nm, and the particles are not biopersistent, they are biodegradable.

Rutin nanocrystals formulations were compared to a cream with a water soluble rutin derivative *in vivo*. The concentration of dissolved active in the water phase was 1/500 in the nanocrystals formulation, but the Sun Protection Factor (SPF) was still higher for the nanocrystal formulation as compared to water soluble derivative (Petersen, 2006). The underlying mechanism of action is: The nanocrystals increase the solubility of the poorly soluble active in the water phase, this leads to an increased concentration gradient between formulation and skin, thus increased penetration compared to micronsized powder. The original molecule rutin is more lipophilic, therefore penetrates better than the hydrophilic derivative. In addition, the original molecule might have more activity in the cell than the derivative. Active penetrated from the water phase into the skin is rapidly replaced by fast dissolving active from the nanocrystals, they act as depot in the water phase (Fig. 5).

The same principle can be applied to pharmaceutical dermal formulations. Diclofenac sodium nanosuspension for transdermal delivery showed increased permeability flux of drug across the skin by up to 3.8 fold compared to the control when tested in Yucatan micropig (YMP) skin model (Piao et al., 2008). Similarly antioxidant activity of hesperetin as nanocrystals was significantly increased when tested *in vitro* by radio scavenging method (Al Shaal et al., 2010b) and can be used as effective adjuvant in skin care or dermatological preparation. Basically the same nanosuspensions can be applied to mucosal surfaces, either as nanosuspension spray, or lotions. The adhesive effect due to the nanosize can further be

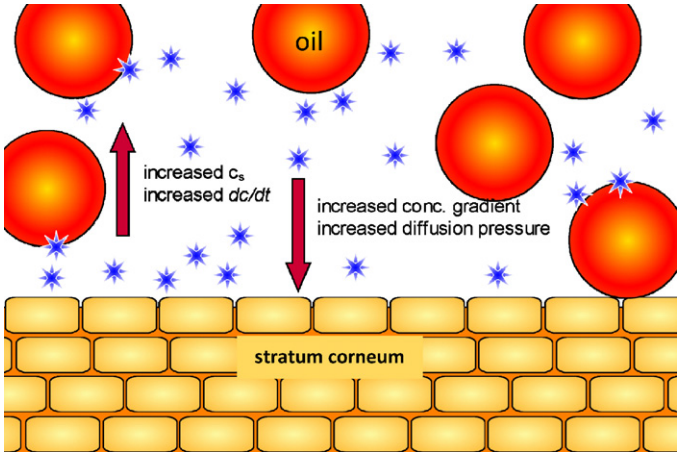

Fig. 5. Mechanism of action of nanocrystals in o/w cream: The nanocrystals (stars) are dispersed in the water phase besides the oil droplets. In the water phase is an increased saturation solubility Cs, leading to an increased concentration gradinet and diffusion pressure into the skin. Penetrated active is fastly replaced in the water phase due to the increased dissolution velocity dc/dt of nanocrystals compared to microcrystals.

enhanced by the use of positively charged polymers as stabilizers for the drug nanocrystals. The opposite charge leads to an increased affinity of the drug nanocrystals to the negatively charged cells (unpublished data). This principle was previously shown by producing antiseptic lipid nanoparticle sprays, using cetylpyridinium chloride as cationic surfactant and antiseptic at the same time (Müller et al., 2009). Ultrafine lidocaine base nanocrystals were prepared as prolonged release formulation for dermal use using the combination technology (Shegokar et al., 2010b). Vaginal and rectal administration can treat local STD (sexually transmitted diseases) by evenly spreading of drug in the local area (Friedrich and Müller-Goymann, 2003).

4.4. Ocular drug delivery

Nanosuspensions have not been yet exploited for this route of drug administration. The general problem is that solutions are relatively fast cleared from the eye, adhesive nanoparticulate suspensions can show prolonged release due to their adhesion properties. Polymeric (Eudragit RS 100 and Eudragit RL 100) nanoparticulate suspensions of flurbiprofen and ibuprofen (Bucolo et al., 2002) revealed superior *in vivo* performance over the existing marketed formulations and could sustain drug release for 24 h. This proves the principle. Another example is acyclovir loaded Eudragit RS polymeric nanoparticle suspensions (Dandagi et al., 2009). Drug nanosuspensions can also be used for drugs that exhibit poor solubility in lachrymal fluids providing advantages of prolonged residence time in a cul-de-sac. Currently there are few studies are investigating NSAIDs in the form of nanocrystals for ophthalmic application (Araújo et al., 2009). An increased rate and extent of drug absorption and intensity of drug action was reported for ocular nanosuspensions of hydrocortisone, prednisolone and dexmethasone (Kassem et al., 2007).

In contrast to the polymeric nanoparticles, nanosuspensions have a clear regulatory advantage. The particles are drained via the lipophilic channels to the nose, from here to the pharynx. That means, the materials used in formulation need to be approved for occular administration. As many polymers are not approved by official authorities. As nanosuspensions do not contain any matrix material, and are purely composed of drug and comparatively small amount of stabilizer. Many stabilizers suitable for stabilization of nanosuspensions are listed in GRAS catalogue (US-FDA).

4.5. Pulmonary drug delivery

As an alternative to dry powders for inhalation, nanosuspensions can be used in case of poorly soluble drugs. Application can simply be performed by placing aqueous nanosuspensions in an aqueous nebulizer, e.g. Pari Boy, or use portable nebulizers on the market. The nebulizer generates an aerosol, with a droplet size suitable for pulmonary administration, e.g. 1–5 μm droplets. The nanocrystals are contained inside these droplets. The nanocrystals cannot be inhaled as a powder. First of all, the nanocrystals are highly adhesive with a tendency to agglomerate, and in addition particles below 0.5–1 μm are being exhaled.

The advantage of nanocrystals is that they show an increased dissolution velocity compared to micronsized crystals. When the aerosol droplets deposit in the lung, as fine particles they should spread more evenly on the lung surface, especially when stabilized with surfactants with good spreadability. Budesonide (corticosteroid) nanosuspensions for pulmonary delivery have been successfully formulated by Müller and Jacobs (2002). It could be shown, that nebulisation with a Pari Boy did not significantly change the size distribution of the nanosuspension. Intensive studies for pulmonary delivery of nanosuspensions were performed by Hernandez-Trejo (2006) by comparing different commercial portable nebulizers regarding their ability to nebulize nanosuspensions (Hernandez-Trejo et al., 2005). All of them were suitable, showing that nebulisation of well-stabilized nanosuspensions can be performed successfully. Fluticasone nanosuspension was delivered as pulmonary aerosol to mice, showing a dose-dependent deposition. The results were found highly repeatable and robust (Chiang et al., 2009).

4.6. Targeted drug delivery

The need to target drugs to specific sites by means of nanoparticles is increasing day by day as a result of therapeutic and economic factors. Nanosuspensions can be used for targeted delivery as their surface properties and *in vivo* behaviour can easily be altered. Their versatility and ease of scale-up enable the development of commercially viable nanosuspensions. The engineering of stealth nanosuspensions (analogous to stealth liposomes) by using various surface coatings for active or passive targeting is the future of targeted drug delivery systems. Targeting of Cryptosporidium parvum (cryptosporidiosis) by using surface-modified mucoadhesive nanosuspensions of bupravaquone was studied by Kayser (Kayser, 2001). A superior targeting was achieved for mucoadhesive bupravaquone nanosuspensions, because of their prolonged residence at infection site. They showed a 10-fold reduction in the infectivity score of Cryptosporidium parvum as compared to the bupravaquone nanosuspensions without mucoadhesive polymers. Similarly, pulmonary aspergillosis can easily be targeted by using amphotericin B nanosuspensions instead of using stealth liposomes (Kohno et al., 1997). The potential to target the brain was already discussed in Section 4.2. Targeting of HIV viral reservoirs was successfully achieved by using bare and coated nevirapine nanosuspensions (Shegokar et al., 2009). In addition, nanosuspensions can be used as supportive treatment in various disease conditions like cancer (Shegokar et al., 2010a), TB and HIV/AIDS.

Important for intravenous targeting is the adjustment of the surface properties of the nanocrystals. The surface properties determine the qualitative and quantitative composition of the adsorption patterns of blood proteins (Blunk et al., 1993). These adsorbed proteins determine subsequently the fate of the injected particles in the body. Fate means recognition by the MPS system and primarily accumulation in liver and spleen, circulation in the blood as stealth particles or enrichment at other sites, e.g. brain or bone marrow. The surface properties can be adjusted such a way, that

the particles even adsorb automatically the blood proteins responsible for enrichment at the desired target site. This is the concept of "differential protein adsorption", which was exploited for targeting nanocrystals to the brain (cf. Section 4.2).

5. Nanocrystals in nutrition

There is an increasing consciousness about nutritional health and an increasing demand to complement the daily nutrition by additives or nutraceuticals. From the philosophy for a healthy population, nutrition plays a very important role. The nutraceutical market is growing, and there are many nutraceutical compounds, e.g. antioxidants, which are poorly soluble. Presently most popular molecules is Coenzyme Q10 capsules, but Q10 has a low oral bioavailability. There are products on the market, claiming to contain "nano Q10" being 100% bioavailable (e.g. containing surfactants for solubilization), requiring only one tenth of the regular dose in normal products. Nanocrystals are also a suitable formulation technology for poorly soluble nutraceuticals like Coenzyme Q10, rutin, hesperidin, apigenin etc. As shown in Fig. 4 and discussed in Section 4.1, the nanocrystals showed superior performance against marketed product when tested for *in vitro* release pattern. It was surprising that in some marketed products e.g. hesperidin, the dissolved percentage of drug after half an hour was still close to zero (Mauludin, 2008). Nanocrystals can be efficiently used to provide effective, bioavailable nutraceutical products in future.

6. Nanotoxicology of nanocrystals

About 5 years ago, nanotechnology was looked at mainly from the positive aspects. In the last 2-3 years there is an increasing concern about potential nanotoxicity of nanosized particles. The public perception is changing from unanimously positive to critical or even major concern, promoted by sometimes unreflected reports in newspapers or newsmagazines. The scientific background for this is, that when moving to the nano size range, physicochemical properties of particles change, giving them also potentially new toxic features. Therefore nanotoxicology is getting an increasingly important role, while developing safe nanocarriers (Holsapple et al., 2005). Discussing about nanotoxicity, it is first important to define: What is a nanoparticle? From the pharmaceutical view, and considering the size dimension, nanoparticles are particles from 1 to 1000 nm. Particles of major toxicological concern are the particles below 100 nm (e.g. FDA, European Cosmetic Regulation (Kislalioglu, 1996). The background is that properties of particles <100 nm are again very much different to large nanometer particles (e.g. 200–800 nm). Example: Large nanometer particles can only be internalized by macrophages (=limited cell number in the body), and cause effects inside the cell. Particles below about 150 nm can be internalized by any cell via pinocytosis. That means these particles can access any cell of the body giving them a higher cytotoxicity risk. In addition, a higher toxic potential is allocated to "biopersistent" nanoparticles, e.g. Fullerenes and carbon nanotubes. They stay forever. Consequently in the new European cosmetic regulation, cosmetic products need to be labeled as nanoproducts when they contain particles which are below 100 nm and biopersistent. Considering these outlines, nanocrystals of poorly soluble compounds can be considered as safe. In most products, they are above 100 nm. In addition, the very important property is that they are biodegradable. After addition of sufficient water, they just dissolve (and this is their purpose in the body). Each drug particle dissolving in the gastrointestinal tract will move from the "μm" to the "nm" size in the dissolution process. Nevertheless, it is important to investigate potential cytotoxic effects, which can occur within the lifetime of a biodegradable nanoparticle. For example, a lifetime might still be sufficient to irritate the immune system. However, considering the aspects above, the nanocrystals are definitely belonging to the nanoparticles with best tolerability, proven also by the number of products on the market.

7. Conclusions

Drug nanocrystals are a promising formulation technology for poorly soluble drugs. Specific solubilization technologies, e.g. cyclodextrins, are only applicable to certain molecules. In case of cyclodextrins, either the molecule fits into the cavity or does not. In contrast, the smartness of nanocrystals is that the technology can be applied to practically any drug, because each drug can be diminuted. It is a general solubilization technology. The industrial applicability is also reflected by the short time between invention and the first products on the market, less than 10 years. This time to the market is not the only criterion. The second one is, if after the first product are more to come. Table 3 shows impressively the number of products in clinical phase, remarkable for such a relatively young technology. Of course, one should apply the simplest technology to solve a formulation and delivery problem. For example, if a poorly soluble oral drug can be formulated as solubilized oily formulation in a gelatin capsule, this would be the formulation of choice. But there are many drugs which are too poorly soluble, and for which the nanocrystals technology can be applied. To make a technology industrially feasible, the excipients required should have a regulatory accepted status, and production on large scale should be possible, which nanocrystals offer. Especially the excipient status is unproblematic, because the particles consist only of drug, and stabilizer–many accepted stabilizers for the different administration routes are available. By now, development focussed mainly on the oral and the intravenous route. There is much more potential for innovative products using the other route as discussed above. For the future, a further increasing use of the nanocrystals technology is predicted, spreading to various administration routes, but also use outside of pharma, e.g. in cosmetics or for bioavailability enhancement of nutraceuticals.

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