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Mini review

Top-down production of drug nanocrystals: Nanosuspension stabilization, miniaturization and transformation into solid products

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ABSTRACT

During the last 10–15 years, the formulation of drugs as nanocrystals has rapidly evolved into a mature drug delivery strategy, with currently five products on the market. The major characteristic of these systems is the rapid dissolution velocity, enabling bioavailability enhancement after oral administration. This mini-review focuses on recent advances with respect to three topics considering drug nanocrystals. The first topic is nanosuspension stabilization. A current literature status is provided and special attention is given to studies attempting to extend our physicochemical understanding of the underlying principles. The second part describes recent advances on miniaturization of nanosuspension production, to enable formulation screening during preclinical development. Finally, literature available on further nanosuspensions solidification is discussed, focussing on the maintenance of the preservation of the rapid dissolution properties of the nanocrystals after further downstream processing.

1. Introduction

Given the increasing number of compounds emerging from discovery programs having poor aqueous solubility and/or dissolution (Lipinski, 2002), pharmaceutical scientists are constantly seeking new formulation approaches in order to obtain an adequate oral bioavailability. Currently, novel possibilities are offered by the rapidly emerging field of nanoscience. An illustration of the increasing interest in nanosciences in general and nanoparticles in particular within the field of pharmaceutical sciences is provided in Fig. 1. Interest in this field began in and rose steadily during the nineties. Since 2000, the number of scientific and patent publications increased dramatically.

One of the nanoscience approaches that has rapidly gained a proven record within the pharmaceutical sciences is the formulation as nanoparticles. These particles have a size below 1 μm, typically a few hundred nanometers (Müller et al., 2006). The particles can be obtained either by particle size reduction of larger crystals, forming nanocrystals (top-down approach) or by building up particles by precipitation of dissolved molecules (bottom-up approach) (Rabinow, 2004). Top-down approaches for drug nanocrystal production comprise high-pressure homogenization and media milling. While the former technique consists of particle size reduction by repeatedly forcing a suspension through a very thin gap (typically about 25 μm) at extremely high velocity, the latter comprises mechanical attrition of suspended drug particles using milling media such as glass (yttrium stabilized) zirconium oxide or highly cross-linked polystyrene resins (Date and Patravale, 2004). Typically, these production processes are conducted in liquid, hence forming a nanosuspension. As the total surface area of the resulting nanosuspension particles is typically orders of magnitude larger compared to a coarse suspension, large quantities of additives may be necessary to ensure adequate stabilization. Therefore, whatever method used for the production of nanosuspensions, a careful evaluation of the type and concentration of the stabilizer used is key to the successful production of nanosuspensions. Both polymeric stabilizers and surfactant stabilizers can be used for this purpose (Rabinow, 2004).

Table 1 summarizes key characteristics of drug nanocrystal products currently on the market. As can be seen from the table, products have been approved by the FDA from the year 2000 on. Second, all five products are based on top-down approaches, four relying on media milling and one on high-pressure homogenization. Although the bottom-up approaches hold tremendous potential with respect to improving bioavailability in obtaining smaller particle sizes (<100 nm) and amorphous drug particles, no commercial application of these systems has yet been realized (Kesisoglou et al., 2007b). A third remarkable point is that all commercial products are intended for oral delivery. This is an illustration of the general preference of the oral route, since it avoids the pain and discomfort associated with injections and is more attractive from a marketing and patient compliance perspective

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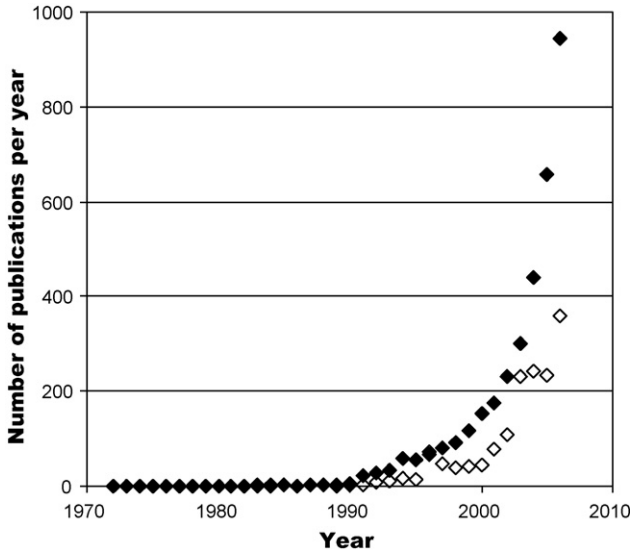

Fig. 1. "Small talk": evolution of the number of scientific (♦) and patent (◊) publications per year on nanoparticles in the pharmaceutical sciences (period 1972-2006): both exhibit a gradual increase in the nineties followed by exponential growth since 2000 [scientific literature source: Science Citation Index Expanded using the query "(nanoparticle* or nanosuspension*) and (drug* or pharmaceutical* or bioactive)" in the title or abstract; patent literature source: esp@cenet®, using the query "A61K (human necessities – medical or veterinary science, hygiene – preparations for medical, dental, or toilet purposes)" for the European classification and "nanoparticle* or nanosuspension*" in the title or abstract; databases were queried on 29/03/2008].

(Fasano, 1998). Furthermore, four of the five products are solids, possible drivers for the latter fact are the convenience of solid dosage forms (marketing aspects) and possible stability issues associated with nanoparticles in their suspended state. These stability issues can be both physical (e.g. Ostwald ripening and agglomeration) and chemical (e.g. hydrolysis), although examples exist for which formulation as a nanosuspension actually prevents the latter, compared with formulation as a solution (e.g. Merisko-Liversidge and Linden, 2003; Müller et al., 2006). Finally, the major advantage of nanocrystals for oral delivery is generally regarded as being a means to increase the dissolution velocity and hence oral absorption, based on the increased specific surface area of the particles. In addition, other advantages such as reduced fasted/fed variability and ease of administration accompany this formulation approach, as denoted in the table.

In view of the above, it is worthwhile to elaborate a bit on the prefix "nano", since it is often the subject of discussion. First, it should be noted that there is currently no consensus on what the term should address (Joachim, 2005). As an example, the definition of nanoscience provided in the UK Royal Society and Royal Academy of Engineering report "Nanoscience, and Nanotechnology: Opportunities and Uncertainties", reads "Nanoscience is the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale." (Royal Society and Royal Academy of Engineering, 2004). For drug nanocrystals, the unique dissolution properties that can be ascribed to the particles, in combination with proven examples of increased bioavailability (Kondo et al., 1993; Liversidge and Conzentino, 1995; Liversidge and Cundy, 1995; Jia et al., 2002; Merisko-Liversidge et al., 2003; Wu et al., 2004; Langguth et al., 2005; Hecq et al., 2006b; Jinno et al., 2006; Hanafy et al., 2007; Kumar et al., 2007) make it correct to classify these systems into the nanotechnology field. On the other hand, more stringent meanings have been ascribed to the prefix, for example the British Standards Institution defines a nanoparticle/nanoparticulate as a "particle with one or more dimensions at the nanoscale", "nanoscale" being defined as "having one or more dimensions of the order of 100 nm or less" (British Standards Institution, 2005). The fact that drug nanocrystals have dimensions typically larger than 100 nm conflicts with this definition as they belong to the colloidal domain. However, nanoparticle production clearly contrasts with micronization. Micronization yields "microparticles" characterized by a mean particle size in the lower micrometer range and used to be the ultimate particle size achievable. During the last 10–15 years, evolutions in particle size reduction processes have made the production of submicron-sized particles possible. Taking this evolution in mind, the wide use of terms as "nanonization", "nanoparticles", "drug nanocrystals" to describe these processes or particles within the pharmaceutical sciences are acceptable. The terms will be used as such in the remainder of this mini-review.

The aim of this mini-review is not to provide an extensive overview on all aspects of drug nanosuspensions. The interested reader is referred to excellent reviews available in the field considering nanosuspensions in general (Müller and Böhm, 1998; Müller et al., 1999a,b, 2000a,b, 2001; Merisko-Liversidge et al., 2003; Müller and Keck, 2004; Patravale et al., 2004; Rabinow, 2004; Rao et al., 2004; Gupta, 2006), their manufacturing techniques (Jahnke, 1998; Horn and Rieger, 2001; Müller et al., 2003; Date and Patravale, 2004; Hu et al., 2004; Keck and Müller, 2006; Müller et al., 2006) and oral or parenteral applications of nanosuspensions (Kipp, 2004; Rabinow and Chaubal, 2006; Kesisoglou et al., 2007a,b; Wong et al., 2008). Rather, the aim of this paper is to provide an update on a number of topics that the authors feel have not received adequate attention. First, nanosuspension stabilization for the pre-

Table 1
Key product characteristics of available commercial products relying on drug nanoparticle technology (Elan, FDA Orange Book, SkyePharma)

Product, active ingredient, company	Date of FDA approval	Manufacturing approach, manufacturing technique	Dose, dosage form	Rationale for development as a nanocrystalline dosage form
Rapamune®, Sirolimus, Wyeth	August 2000	Top-down, media milling	1 and 2 mg, tablets	Reformulation of the oral solution that requires refrigeration storage and is less easy to administer.
Emend®, Aprepitant, Merck	March 2003	Top-down, media milling	80 and 125 mg, capsules	New chemical entity, formulation as nanocrystals reduces fed/fasted variability.
TriCor®, Fenofibrate, Abbott	November 2004	Top-down, media milling	48 and 145 mg, tablets	Reformulation for a more flexible dosing regime and to prevent the need of administration with a meal
Megace® ES, Megestrol acetate, Par Pharmaceutical	July 2005	Top-down, Media milling	125 mg/ml, nanosuspension	Reformulation of the oral suspension to obtain a higher dissolution rate, bioavailability and ease of administration (reduced dosing volume and suspension viscosity).
Triglide®, Fenofibrate, Skye Pharma	May 2005	Top-down, high-pressure homogenization	50 and 160 mg, tablets	Reduce fed/fasted variability, as for TriCor®.

vention of nanoparticle agglomeration is reviewed. Second, in view of the current importance of formulation miniaturization for preclinical formulation screening purposes, a synthesis of the available literature is provided. Finally, given the importance of further transformation of nanosuspensions into solids, solidification of drug nanosuspensions is discussed.

2. Nanosuspension stabilization

2.1. Stabilization principles

The manufacturing of a nanosuspension implies the creation of additional surface area and hence interface. As the Gibbs free energy change, associated with the formation of additional interface is positive, the nanosuspensions formed are thermodynamically unstable and will tend to minimize their total energy by agglomeration (Gonzáles-Caballero and de Dios Garciá López-Durán, 2000). Kinetically, the process of agglomeration depends on its activation energy. This activation energy can be influenced by adding stabilizers to the system. A first requirement for a stabilizing system is that it provides wetting of the hydrophobic surfaces of the drug particles. Additionally, the stabilizers should prevent agglomeration of the nanoparticles, by increasing the activation energy of the process. In other words, an adequate stabilizer should provide a barrier to agglomeration. Possible mechanisms for providing this barrier are electrostatic and steric stabilization which can be obtained by adding charged surfactants and non-ionic surfactants/polymers, respectively. Theoretically, the concept of an energetic barrier can be understood by the (classical/extended) DLVO theories (Derjaguin and Landau, 1941; Verwey and Overbeek, 1948; van Oss et al., 1990; Israelachvili, 1992; van Oss, 1994; Durán et al., 1995), describing the interaction of solid particles in a liquid medium in terms of (i) attractive, Lifshitz-van der Waals interactions, (ii) repulsive, electrostatic interactions between the electric double layers surrounding the particles in solution and (iii, extended) solvation, or structural forces coming from the more or less structured layer of solvent molecules around the solid particles that can be attractive (hydrophobic particles) or repulsive (hydrophilic particles). The stabilizer exerts its effect through its influence on the different components contributing to the overall interaction. An illustrative example of the total free energy of interaction as a function of interparticle distance (classical DLVO theory, taking into account only the first two contributions of the total interaction) is provided in Fig. 2. In this case, the total potential energy (V_{tot}) is the sum of the (repulsive) electrostatic energy (V_{el}) and the (attractive) Lifshitz-van der Waals energy (V_{lw}). The concept of electrostatic stabilization can be understood by its influence on V_{el}. For steric stabilization, influences on the free energy of interaction are less straightforward to interpret. However, it can be understood that, as two particles surrounded by an adsorbed polymer layer approach each other, there will be a local increase in polymer concentration in that region. The latter can result in a (local) increase in osmotic pressure, yielding a positive contribution to V_{tot}.

2.2. Literature examples

Table 2 provides an overview of scientific literature examples for which nanosuspension production was successful. Examples from patent literature were not included, since list is too extensive (for example, Elan's NanoCrystal™ technology has been protected by over 700 patents). A first remark on these data is that the group of stabilizers typically selected for nanosuspension production is rather limited. This is in contrast with the number of potential stabilizers that could be applied for the purpose [see e.g. Rowe et al., 2003 (in general) and Marti-Mestres and Nielloud, 2000 (for surfactants)]. Second, stabilizers are being applied either alone or in combination. The most popular non-ionic surfactants applied are the poloxamers and Tween® 80; sodium lauryl sulfate is the typical ionic surfactant used for this purpose. Additionally, natural biological surfactants such as lecithins and cholic acid derivatives are frequently applied. Polymers used include cellulosics (HPMC, HPC) and polyvinyl alcohol. Numerous patent examples describing the use of povidones can also be found. The drug/stabilizer ratios (w/w) in the formulations vary widely, ranging from 1:3 to 50:1.

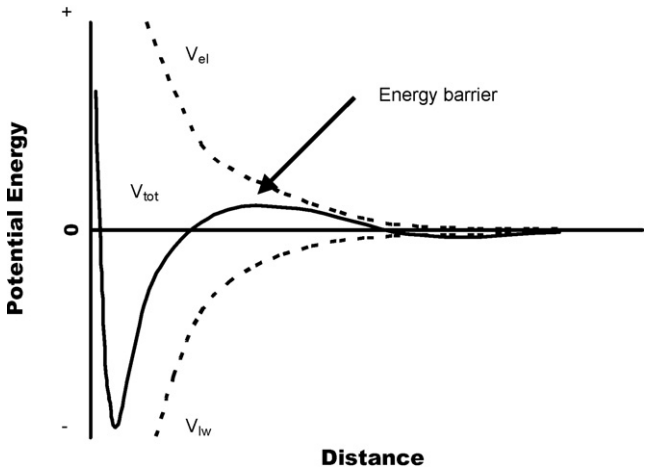

Fig. 2. Illustration of the potential energy as a function of interparticle distance (classical DLVO): total potential energy (V_{tot}), electrostatic energy (V_{el}) and Lifshitz-van der Waals energy (V_{lw}).

Although the number of articles describing nanosuspension formulations is extensive, very little literature is currently available that aims to (i) evaluate and compare the ability of different stabilizers in their stabilizing potential from a more fundamental point of view or (ii) consider the influence of the drug compound with respect to the ease of nanosuspension stabilization. An exception to this is the work from Lee and Choi on polymeric stabilizers. In a first study (Lee et al., 2005), they designed a number of amino acid copolymers with lysine as a hydrophilic segment and phenylalanine, leucine or alanine as the hydrophobic segment. Copolymers were varied in molecular weight (5–25 kDa), chain architecture (random/diblock), lysine content and lysine-hydrophilic amino acid combination. The performance of the stabilizers was evaluated in terms of particle size obtained upon wet comminution using naproxen as a model compound. They concluded that hydrophobicity of the copolymers, rather than molecular weight and chain architecture, was determining the performance of the stabilizer. The less hydrophobic lysine–alanine proved to be unsuccessful. For lysine–phenylalanine and lysine–leucine combinations, the mole fraction of hydrophobic moieties needed to be at least 15 mol% for adequate stabilization. Hydrophobicity was interpreted as necessary to obtain stable polymer adsorption onto the hydrophobic drug surfaces. In a subsequent smaller study (Choi et al., 2005), the ability of povidone (PVP) and hydroxypropylcellulose (HPC) to obtain nanosuspensions for 7 model compounds by wet comminution was evaluated. They tried to relate the particle size results with the surface energy of the model drugs and the stabilizers, determined by contact angle measurements. For HPC, surface energy seemed not to be a dominant factor. For PVP, on the other hand, surface energy could to some extent explain the obtained results. Better nanosuspensions were produced when surface energy values of drug and stabilizer were comparable. Finally, in a recent paper (Lee et al., 2008), they reported on a screening of five polymers [HPC, PVP,

Table 2
Overview of examples of stabilizing systems for nanosuspensions found in scientific literature.

Stabilizing system (% w/w to compound)	Compound (% w/v or w/w in suspension)	MM/HPH[a]	Literature reference
1. Single stabilizer systems			
1.1. Surfactants			
Cremophor® EL (100%)	1,3-Dicyclohexyl urea (1%)	MM	Wahlstrom et al. (2007), Chiang et al. (2007)
Cremophor® RH 40 (12.5%)	Albendazole (4%)	HPH	Kumar et al. (2008)
Lecithin (6.7%)	RMKP 22 (9%)	HPH	Müller and Peters (1998)
Lecithin (20%/40%/167%)	RMKP 22 (3%)	HPH	Peters et al. (1999)
Poloxamer 188 (12.5%)	Albendazole (4%)	HPH	Kumar et al. (2007), Kumar et al. (2008)
Poloxamer 188 (30%/100%)	Azithromycin (1%)	HPH	Zhang et al. (2007)
Poloxamer 188 (20%)	Buparvaquone (2.5%)	HPH	Müller and Jacobs (2002)
Poloxamer 188 (100%)	Buparvaquone (1%)	HPH	Hernández-Trejo et al. (2005)
Poloxamer 188 (4%)	Dexamethasone (2.5%)	HPH	Kassem et al. (2007)
Poloxamer 188 (4%)	Hydrocortisone (2.5%)	HPH	Kassem et al. (2007)
Poloxamer 188 (60%)	Naproxen (5%)	MM	Liversidge and Conzentino (1995)
Poloxamer 188 (10%-100%)	Omeprazole (1%-10%)	HPH	Möschwitzer et al. (2004)
Poloxamer 188 (4%)	Prednisolone (2.5%)	HPH	Kassem et al. (2007)
Poloxamer 338 (50%)	Camtothecin (2%)	MM	Merisko-Liversidge et al. (1996)
Poloxamer 338	Itraconazole	MM	Mouton et al. (2006)
Poloxamer 407	301029	MM	Jia et al. (2002)
Poloxamer 407 (50%)	Danazol (1%)	HPH	Crisp et al. (2007)
Poloxamer 407 (50%)	Etoposide (2%)	MM	Merisko-Liversidge et al. (1996)
Poloxamer 407 (20%)	Iodipamide (5%/7.5%/15%/20%/30%)	MM	Zheng and Bosch (1997)
Poloxamer 407 (50%)	Itraconazole (1%)	HPH	Crisp et al. (2007)
Poloxamer 407 (50%)	Paclitaxel (2%)	MM	Merisko-Liversidge et al. (1996)
Poloxamer 407 (2%)	ucb-35440-3 (5%)	HPH	Hecq et al. (2006a)
Poloxamine 908 (20%)	Ethyl diatrizoate (20%/30%)	MM	Na et al. (1999)
Polyglyceryl-10 laurate (16.7%)	HO-221 (30%)	MM	Kondo et al. (1993)
Sodium lauryl sulfate (12.5%)	Albendazole (4%)	HPH	Kumar et al. (2008)
Sodium lauryl sulfate (5%)	Spironolactone (10%)	HPH	Langguth et al. (2005)
Tween® 80 (12.5%)	Albendazole (4%)	HPH	Kumar et al. (2008), Kumar et al. (2007)
Tween® 80	Atovaquone	HPH	Schöler et al. (2001)
Tween® 80 (30%)	RMKP 22 (1%)	HPH	Grau et al. (2000)
Tyloxapol (20%)	Beclomethasone dipropionate (10%)	MM	Ostrander et al. (1999)
Tyloxapol (50%)	Budenoside (1%)	HPH	Jacobs and Müller (2002)
1.2. Polymers			
Acacia gum (2%)	ucb-35440-3 (5%)	HPH	Hecq et al. (2006a)
HPC (60 kDa; 16.7%)	Undisclosed (1.8%/6.1%/11.5%)	MM	Lee and Cheng (2006)
HPC (60 kDa; 2.4%/4.8%/9.6%/19.3%)	Undisclosed (16%)	MM	Lee (2003)
HPMC (Methocel E15; 10–200%)	Nifedipine (5%)	HPH	Hecq et al. (2006b)
HPMC (Methocel E15; 2%)	ucb-35440-3 (5%)	HPH	Hecq et al. (2006a)
Polyvinyl alcohol (30–70 kDa; 50%)	Beclomethasone dipropionate (5%)	MM	Wiedmann et al. (1997)
Polyvinyl alcohol (13–23 kDa; 2%)	ucb-35440-3 (5%)	HPH	Hecq et al. (2006a)
Povidone K15 (30%)	Danazol (5%)	MM	Liversidge and Cundy (1995)
2. Multiple stabilizer systems			
2.1. Surfactant combinations			
Lecithin (20%)–Sodium cholic acid (16.7%)	Prednisolone (3%)	HPH	Müller and Peters (1998)
Lecithin (20%)–Sodium cholic acid (16.7%)	RMKP 22 (3%)	HPH	Müller and Peters (1998)
Lecithin (20%)–Sodium cholic acid (16.7%)	RMKP 23 (3%)	HPH	Müller and Peters (1998)
Lecithin (50%)– Tyloxapol (20%)	Budenoside (1%)	HPH	Jacobs and Müller (2002)
Poloxamer 188 (20%)–Lecithin (10%)	Azithromycin (1%)	HPH	Zhang et al. (2007)
Poloxamer 188 (10–100%)–Lecithin (5–50%)	Bupravaquone (1–10%)	HPH	Jacobs et al. (2001)
Poloxamer 188 (100%)–Lecithin (50%)	Buparvaquone (1%)	HPH	Müller and Jacobs (2002)
Poloxamer 188 (7.5%)–Lecithin (2.5%)	Oridonin (5%)	HPH	Gao et al. (2008)
Poloxamer 188 (25%)–Lecithin (30%)–Sodium cholic acid (12.5%)	Clofazimine (2%)	HPH	Peters et al. (2000)
Poloxamer 188 (120%)–Sodium cholic acid (80%)	Nimodipine (0.5%)	HPH	Xiong et al. (2008)
Poloxamer 188 (62.5%)–Sodium cholic acid (12.5%)–Tween® 80 (125%)	Amphotericin B (0.4%)	HPH	Kayser et al. (2003)
Poloxamer 18–Sodium deoxycholic acid	Itraconazole	HPH	Rabinow et al. (2007)
Poloxamer 188 (50%)–Sodium deoxycholic acid (5%)	Zn-Insulin (2%)	MM	Merisko-Liversidge et al. (2004)
Poloxamer 188 (12.5%)–Sodium lauryl sulfate (12.5%)	Albendazole (4%)	HPH	Kumar et al. (2008)
Poloxamer 188 (12.5%)–Tween® 80 (12.5%)	Albendazole (4%)	HPH	Kumar et al. (2008)
Poloxamer 188 (50%)–Tween® 80 (50%)	Loviride (17%)	MM	Van Eerdenbrugh et al. (2007)
Poloxamer 188 (5%/10%)–Tween® 80 (2.5%/5%)	Tarazepide (10%)	HPH	Jacobs et al. (2000)
Poloxamer 188–Tween® 80–Sodium cholic acid	Atovaquone	HPH	Schöler et al. (2001)
Tween® 80 (20%)–Lecithin (10%)	Azithromycin (1%)	HPH	Zhang et al. (2007)
Tween® 80 (2.5–5%)–Potassium oleate (5–10%)	RMKK99 (10%/20%/30%)	HPH	Krause and Müller (2001)

Table 2 (*Continued*)

Stabilizing system (% w/w to compound)	Compound (% w/v or w/w in suspension)	MM/HPH[a]	Literature reference
Tween® 80 (12.5%)–Sodium lauryl sulfate (12.5%)	Albendazole (4%)	HPH	Kumar et al. (2008)
Tween® 80 (16.7%)–Span 80 (33.3%)	Piposulfan (2%)	MM	Merisko-Liversidge et al. (1996)
2.2. Polymer—surfactant combinations			
Carbopol 974 P (2.5%)–Tween® 80 (12.5%)	Albendazole (4%)	HPH	Kumar et al. (2008)
HPC–Sodium lauryl sulfate	Cilostazol	MM	Jinno et al. (2006)
HPC (80%)–Sodium lauryl sulfate (1.6%)	MK-0869 (5%)	MM	Wu et al. (2004)
HPMC (K4MCR; 12.5%)–Tween® 80 (12.5%)	Albendazole (4%)	HPH	Kumar et al. (2007)
Polyvinyl alcohol (50%)–Poloxamer 188 (50%)	Buparvaquone (1%)	HPH	Hernández-Trejo et al. (2005)
Polyvinyl alcohol (100%)–Poloxamer 188 (200%)	Buparvaquone (1%)	HPH	Müller and Jacobs (2002)
Povidone K15 (11.3%)–Sodium lauryl sulfate (0.57%)	AZ68 (10%)	MM	Sigfridsson et al. (2007)
PVP VA (23.3%)–Sodium lauryl sulfate (1.67–3.33%)	Undisclosed (15%)	MM	Deng et al. (2008)

[a] MM = Media milling, HPH = High-pressure homogenization.

poloxamer 407, polyethylene glycol (PEG) and poloxamer 188] and 11 model drugs for nanosuspension stabilization. In addition, the performance of the polymeric stabilizers in combination with the ionic surfactants sodium lauryl sulfate and benzethonium chloride (added 1 wt% relative to the polymer weight) was investigated. For the polymeric stabilizers alone, they interpreted the poor results obtained for PEG as a result of the lack of hydrophobic units in the polymer to enable adsorption onto the drug surfaces. For the other four polymers, nanosuspension production was successful for five drugs having similar surface energies as the polymers. Additionally, poloxamer 188 was able to stabilize most of the other model compounds. The better performance compared to poloxamer 407 was suggested to originate from the lower molecular weight. Although a polymer with a lower molecular weight is less physically adsorbing, it enables a less kinetically restricted adsorption process (a similar molecular weight trend was also reported for the case of itraconazole stabilized with 7 HPC types). The effect of adding ionic surfactants to the polymer systems on the obtained particle size was variable. The authors also analyzed the obtained results in terms of the properties of the drug compounds studied. Drugs with lower aqueous solubility, higher molecular weight and higher melting point showed to be better candidates for nanosuspension production (i.e. easier to stabilize). However, the fact that only five different polymers were tested for each drug compound, combined with the identification of three, sometimes interrelated, drug properties as being critical for the outcome, makes the analysis somewhat over-interpreted.

In a recent study of our group (Van Eerdenbrugh et al., submitted for publication-a), we evaluated nanosuspension production with 13 stabilizers of different classes, each used in 3 concentrations. Media milling was performed for each of these stabilizing systems for 9 model compounds of structurally different classes using a planetary mill. In general, applying higher stabilizer concentrations [1–10 wt% for the semi-synthetic polymers and 10–100 wt% for the other stabilizers evaluated (relative to the drug weight)] had a positive effect on nanosuspension production and subsequent stability. Semi-synthetic polymers (hydroxypropylmethylcellulose, methylcellulose, hydroxyethylcellulose, HPC, sodium carboxymethylcellulose, sodium alginate) displayed a rather poor stabilizing performance (10% success rate on average, i.e. one out of ten formulations resulted in a nanosuspension), partly due to the viscosity-limited concentration in which they can be prepared. The linear synthetic polymers PVP K30 and PVP K90 showed a better stabilizing potential when applied in higher concentrations (up to a success rate of 56% for PVP K30 in a 100 wt% concentration, relative to the drug weight), an effect that was even more pronounced for the synthetic copolymers poloxamer 188 and Kollicoat IR® (both 67% success rate in a 100 wt% concentration). The effect of a lower molecular weight of PVP yielding higher success rates coincided with the observations on poloxamer and HPC described above. Finally, the surfactants Tween® 80 and TPGS showed the best stabilizing performance. Whereas Tween® 80 applied in concentrations of 25 and 100 wt% of the drug weight had a success rate of 89%, TPGS proved to be a universally applicable stabilizing system in these concentrations. Upon investigating the cause of the large discrepancies observed in the % success rate of nanosuspension production between the different compounds (15–69% success rate), typical physicochemical properties such as molecular weight, melting point, logP, aqueous solubility and density were unable to explain the results. Investigation of the surface properties of nanosuspensions of the different compounds containing 25 wt% TPGS showed that surface hydrophobicity (as illustrated by the amount of TPGS adsorbed per unit of surface area) was able to explain the tendency of nanoparticle agglomeration and hence the probability of successful nanosuspension production.

3. Miniaturization of nanosuspension production

Early identification of enabling formulation approaches helps to guide molecules through preclinical development. Traditionally, formulation efforts are situated during development stages, where compound availability is relatively large. In the preformulation stage and during late discovery, in contrast, compound availability is scarce. Therefore, formulation development in these stages should be performed on minute amounts of drug compound, preferentially in a screening approach. During the last years, an increased interest in the downscaling of different formulation strategies can be noted, illustrated by reports published on the matter (e.g. Chen et al., 2003; Gardner et al., 2004a,b; Dai et al., 2007; Mansky et al., 2007; Shanbhag et al., 2008) and commercial activity developed around it (e.g. Avantium; Symyx; TransForm Pharmaceuticals).

For nanosuspension process development purposes using high-pressure homogenization, the Avestin EmulsiFlex-B3 has been described with a volume of 3.5 ml (Müller et al., 2001). For media milling, the Nanomill® System has been reported (Kesisoglou et al., 2007b). Again, the working capacity of the smallest chamber is still 10 ml. These volumes make screening studies difficult when compound supply is low and production of large numbers of different formulations in parallel is impossible with these devices. The lack of a sufficiently downscaled system to support discovery was acknowledged in the latter reference. The fact that high-pressure homogenization relies on the forcing of a suspension through a small gap makes miniaturization of this technology less straightforward. Media milling, on the other hand, can be per-

formed by agitation of devices containing the starting suspension and milling media. Furthermore, nanosuspension production by media milling is characterized by its ease of scale-up (Date and Patravale, 2004), making results generated on nanosuspensions in downscaled designs valuable.

When looking into literature, we found only two references in patent literature on the downscaling of media milling (Cunningham et al., 2004; Hansell, 2005). The first document describes media milling on milligram to microgram amounts of drug compound. In the examples provided in this document, milling is performed in 24 or 48 well plates. The lowest amount of suspension discussed in the examples of the second patent is 0.5 g. In both patents, the focus lies on the production of nanosuspensions and unfortunately, a thorough physicochemical characterization on these small amounts of nanosuspensions is not discussed. However, for a downscaled process, production of minute amounts of nanosuspension should be complemented with a thorough physicochemical characterization on small amounts of sample. Examples of important physicochemical evaluations of nanosuspensions and/or nanoparticles include size, drug content, morphology, thermal characteristics and X-ray powder diffraction. In a parallel screening design, such a process would enable a rapid optimization of nanosuspension production for compounds in preclinical development. Examples of screening parameters that can be evaluated include the drug content, stabilizer type, stabilizer content and type and amount of milling material used for nanosuspension production.

In a recent study (Van Eerdenbrugh et al., submitted for publication-c), we explored scaling down production (by media milling) and evaluation of nanosuspensions. For nanosuspension production, two different milling designs were evaluated that allow parallel production of nanosuspensions for screening purposes. Amounts of drug compound used for nanosuspension production were as low as 10 mg per experiment, an amount of drug on which a thorough physicochemical characterization is still possible. Scaling down of the nanosuspension production process by media milling using amounts as low as 10 mg of drug compound was feasible for seven model drug compounds tested in a 96 well plate design. Although the 96 well plate can be easily integrated into high throughput settings, well deformation or wear observed in the study might limit its practical use. To circumvent this problem, milling in glass vials in a ball mill was evaluated as an alternative, as medium and low-energy mills have previously been described for their capability in obtaining nanosuspensions (e.g. Liversidge and Conzentino, 1995; Liversidge and Cundy, 1995; Merisko-Liversidge et al., 1996; Lee, 2003; Lee and Cheng, 2006; Van Eerdenbrugh et al., 2007). Again, scaling down was feasible for 10 mg of drug compound and results obtained were comparable to those obtained in the 96 well plate design. As a miniaturization of nanosuspension characterization goes hand in hand with a downscaled production process, feasibility of performing evaluations on amounts of nanosuspension corresponding to 1 mg was investigated. Drug content evaluation was precise and accurate, enabling transfer of minute amounts in a quantitative manner. For size measurement in a miniaturized way, dynamic light scattering was identified as the method of choice. For morphology evaluation by SEM, data quality depended to a large extent on the method of sample preparation. Thermal analysis (DSC) was feasible by different sample preparation techniques, although the preparation method applied was found to impact the melting behavior of the stabilizer. Further solid state characterization of the nanoparticles by X-ray powder diffraction in capillaries was possible. Measuring freeze-dried nanosuspension yielded superior results, compared to directly measuring the nanosuspension in the capillary. The latter, however, can be used as a fingerprint for solid-state identification of the drug compound after milling. Summarizing, both production and a thorough physicochemical characterization of amounts of nanosuspension containing as low as 10 mg of drug compound was found to be feasible. Concluding, the approach presented proved promising for nanosuspension formulation screening studies in parallel designs, a valuable tool in preclinical development settings. However, it should be born in mind that characteristics such as obtained particle size and solid state could differ between small scale experiments and large scale production, due to differences in energy input and particle size reduction mechanisms in different milling designs.

4. Further transformation into solid products

For oral administration, the rapid dissolution originating from the increased specific surface area of drug nanocrystals is generally regarded as its main advantage. In the suspended state, this can be achieved by the selection of a proper stabilizing system, preventing nanoparticle agglomeration, as discussed in Section 2. However, as highlighted in the introduction, further transformation into solid products is often required for physical stability and/or patient convenience reasons. Technically, transformation of nanosuspensions into solid products can be achieved using established unit-operations such as freeze-drying, spray-drying, pelletization and granulation (Müller et al., 2006). The obtained powders could be used as such, e.g. as a powder for reconstitution or as rapidly disintegrating freeze-dried single dosage forms. Alternatively, further processing steps such as capsule filling or compression into tablets can be performed. During all these steps, maintenance of the rapid dissolution characteristics of the nanoparticles is imperative and should be evaluated. Wetting and disintegration characteristics of the products upon addition of the solid dosage form to water should be good in order to maintain these dissolution characteristics. Therefore, often a matrix former is added to the suspension prior to the drying operation. Typical matrix formers added prior to drying are water-soluble sugars, as adapted from freeze-drying (Kesisoglou et al., 2007b).

Table 3 summarizes the examples found in literature on solidification of nanosuspensions. Prior to discussing the results of these studies, it should be stressed that the stabilizers used during production can have an effect on the redispersability of the dried product. Albendazole serves as a nice example to illustrate this point. In contrast to formulations containing HPMC or carbopol as a nanosuspension stabilizer, agglomeration was observed when other nanosuspension stabilizers were used.

Most examples provided in the table are based on freeze-drying or spray-drying. As can be seen from the table, mannitol and sucrose are very popular as matrix formers for these purposes. For freeze-drying, their performance relies partly on their cryoprotective action. An example of the cryoprotective action is given for compound AZ68, where the addition of mannitol prior to freezing is necessary to prevent agglomeration. In the study by Lee and Cheng (Lee and Cheng, 2006), the importance of the freezing rate applied as well as the drug concentration of the nanosuspension that was frozen was demonstrated. Higher freezing rates and lower drug concentrations resulted in less agglomerated products. Clear examples where the final dried product showed good reconstitution characteristics due to the inclusion of sugars are Danazol (sucrose and to some extent mannitol, freeze-drying), Loviride (sucrose, freeze-drying) and Nifedipine (mannitol, spray-drying). An example where this was clearly not the case is that of itraconazole freeze-dried with sucrose (Van Eerdenbrugh et al., 2008b); although the cryoprotective effect could be observed, agglomeration occurred during the last phase of the drying step. Unexpectedly, this became more pronounced upon using higher

Table 3
Overview of examples of nanosuspension solidification found in literature.

Technology	Compound	MM/HPH[a]	Matrix formers (%wt relative to drug)	Evaluation(s) and result(s)	Literature reference
Cooling (freezer/refrigerator)[b]	AZ68	MM	None Mannitol (2632 wt%)	Refrigerator: No mannitol: no agglomeration (DLS[c]) Mannitol: no agglomeration (DLS[c]) Freezer: No mannitol: agglomeration Mannitol: no agglomeration, cryoprotective (DLS[c])	Sigfridsson et al. (2007)
Freeze-drying	Albendazole	HPH	Mannitol (25, 125, 250 wt%) Sucrose (250 wt%)	Without additional HPMC or carbopol: Agglomeration With additionally 12.5 wt% HPMC or 2.5 wt% carbopol: No agglomeration (DLS[c])	Kumar et al. (2008)
Freeze-drying	Azithromycin	HPH	None	Dissolution compared to micronized powder: 65% vs. 20% dissolved in 5 h Still rather poor dissolution characteristics	Zhang et al. (2007)
Freeze-drying	Clofazimine	HPH	Mannitol plus: (280 wt%) None Trehalose (100, 400 wt%) Mannitol (400 wt%)	Reconstituted powders (manual shaking, 2 min) have: A size comparable to that prior to drying (DLS[c]) An $d_{v;99\%}$ comparable to that prior to drying (LD[d])	Peters et al. (2000)
Freeze-drying	Danazol	MM	PVP plus: (30 wt%) None Tween® 80 (0.4 wt%) Mannitol (40 wt%) Sucrose (40 wt%)	Upon reconstitution, number of particles larger than: 10 μm: None 17063; Tween® 80 18148; Mannitol 19196; Sucrose 6368 30 μm: None 2153; Tween® 80 3071; Mannitol 77; Sucrose 94 80 μm: None 1; Tween® 80 2; Mannitol 0; Sucrose 0 100 μm: None 0; Tween® 80 0; Mannitol 0; Sucrose 0	Liversidge et al. (1994)
Freeze-drying	Itraconazole	MM	None Sucrose (50, 100, 200 wt%) Microcrystalline Cellulose (50, 100, 200 wt%)	Sucrose: Higher amounts of sucrose result in decreased dissolution rates Although sucrose acts cryoprotective, it triggers agglomeration during the last phase of the drying step Microcrystalline cellulose: Higher amounts of microcrystalline cellulose result in increased dissolution rates	Van Eerdenbrugh et al. (2008b)
Freeze-drying	Loviride	MM	None Sucrose (100 wt%)	No sucrose: Agglomeration (DLS[c]) Only 58.1 ± 26.3% dissolution after 15 min Sucrose: No agglomeration (DLS[c], LD[d]) Complete dissolution within minutes	Van Eerdenbrugh et al. (2007)
Freeze-drying	Naproxen	MM	None	No agglomeration (DLS[c], short sonication)	Ain-Ai and Gupta (2008)
Freeze-drying	Oridonin	HPH	Mannitol (20 wt%)	103.3 ± 1.5 nm nanosuspension (DLS[c]): dissolution: 93.2% dissolved after 5 min 99.9% dissolved after 10 min 897.2 ± 14.2 nm nanosuspension (DLS[c]): dissolution: 35.4% dissolved after 5 min 75.2% dissolved after 10 min	Gao et al. (2008)
Freeze-drying	Oridonin	HPH	Mannitol (100 wt%)	Dissolution enhanced: 98% dissolved after 24 min 40.3% after 2 h for commercial powder	Gao et al. (2007)

Freeze-drying	Undisclosed	MM	None	Agglomeration upon redispersion (LD^d): Agglomeration suppressed above a certain critical freezing rate Critical freezing rate increases with concentration	Lee and Cheng (2006)
Freeze-drying and spray-drying	9 drug compounds	MM	None	For the six compound where dissolution could discriminate between milled and unmilled products: For all compounds, spray-drying and freeze-drying in general gave similar dissolution results For compounds with more hydrophobic surfaces, dissolution decreased more due to the drying step	Van Eerdenbrugh et al. (2008a)
Spray-drying	3 drug compounds	MM	Microcrystalline	The matrix former performance in terms of the preservation of high dissolution rates showed the rank order: anhydrous dicalcium phosphate < microcrystalline cellulose < colloidal silicon dioxide < hydrophobically modified inuline	Van Eerdenbrugh et al. (submitted for publication-b)
			Cellulose (100 wt%) Anhydrous dicalcium phosphate (100 wt%) Colloidal silicon dioxide (100 wt%) Hydrophobically modified inuline (100 wt%)		
Spray-drying	Amphotericin B	HPH	PVP (50, 500 wt%)	Upon redispersion in water: 500 wt% PVP: no aggregation detectable (LD^d) 50 wt% PVP: small aggregation peak (LD^d)	Müller et al. (2006)
Spray-drying	Cilostazol	MM	None	Dissolution in water, FaSSIF and FeSSIF: Complete within minutes for NanoCrystal® product Significantly slower for jet-milled and hammer milled	Jinno et al. (2006)
Spray-drying	Nifedipine	HPH	None	No mannitol: $d_{(v;0.5)}$ 3.70 ± 0.09 μm; $d_{(v;0.9)}$ 8.60 ± 0.33 μm (LD^d) Dissolution of 20% after 2 min	Hecq et al. (2005)
			Mannitol (100 wt%)	Mannitol: $d_{(v;0.5)}$ 0.339 ± 0.006 μm; $d_{(v;0.9)}$ 1.60 ± 0.04 μm (LD^d) Dissolution of 75% after 2 min	
Spray-drying	Undisclosed	MM	None	Bimodal particle size distribution (LD^d): Submicron peak and peak around 10 μm Slow disintegration: 10 μm disappears after 25 h	Lee (2003)
Spray-coating on sugar spheres	Hydrocortisone Acetate	HPH	None	Controlled release products Eudragit® L 30 D-55 top coating Dissolution: after 2 h pH shift from acidic to 6.8 Faster for submicron product than for micronized Still controlled release, no burst release	Möschwitzer and Müller (2006)
Pelletization of the spray-dried powder in a high shear mixer	Ketoconazole	MM	Pellet formulation	Controlled release formulation	Vergote et al. (2001)
Tabletting after pelletization of the spray-dried powder in a high shear mixer	Ketoconazole	MM	Pellet formulation	Controlled release formulation: Pellets: *in vivo* (dogs): Nanocrystalline: t_{max}: 1.0 h, $t_{75\%Cmax}$: 1.0 ± 0.3 h Microcrystalline: t_{max}: 2.0 h, $t_{75\%Cmax}$: 2.2 ± 0.3 h Compressed pellets: *in vivo* (dogs): Nanocrystalline: t_{max}: 6.0 h, $t_{75\%Cmax}$: 5.6 ± 0.6 h Microcrystalline: t_{max}: 6.0 h, $t_{75\%Cmax}$: 5.4 ± 0.5 h	Vergote et al. (2002)

Table 3 (*Continued*)

Technology	Compound	MM/HPH[a]	Matrix formers (%wt relative to drug)	Evaluation(s) and result(s)	Literature reference
Tabletting by direct compression after spray-drying	Naproxen	MM	Final formulation Naproxen (46.7 wt%) PVP (3.5 wt%) HPC (11.6 wt%) Lactose (35.0 wt%) Mg-stearate (0.23 wt%) Orange coat (3.0 wt%)	Hardness: between 10 and 13 kPa (Aleve®: 14.4) Disintegration time: 2.0–2.5 min (Aleve®: 26) *In vivo* study, fed and fasted dogs: The time to peak plasma concentration (t_{max}) was shorter, compared to the Aleve® formulation There was less variability in rate of absorption, compared to the Aleve® formulation There was reduced fed-fasted difference, compared to the Aleve® formulation	Liversidge et al. (1998)
Tabletting by compression after spray-drying, roller compaction and granulation			Final formulation Naproxen (65.8 wt%) HPC (6.6 wt%) Na CMC (14.8 wt%) Lactose (12.3 wt%) Mg-stearate (0.49 wt%)	Hardness: between 6 kPa (Aleve®: 14.4) Disintegration time: 1.6 min (Aleve®: 26) Dissolution, pH6: 80% release: 7 min (Aleve®: 11)	

[a] MM = Media milling, HPH = High-pressure homogenization.
[b] Although this is not an example of a solidification process, it provides interesting information on the effect of freezing on agglomeration. Since freezing is an essential step in the freeze-drying process, this example was included.
[c] DLS = Dynamic Light Scattering.
[d] LD = Laser Diffractometry.

sucrose amounts. In the same study, the water-insoluble microcrystalline cellulose (Avicel®PH101) proved to be a better matrix former for freeze-drying of the itraconazole nanosuspensions. In a subsequent study, we evaluated the effect of freeze-drying and spray-drying without additional matrix formers for a set of nine compounds (Van Eerdenbrugh et al., 2008a). For three of the compounds (griseofulvin, mebendazole, naproxen), the dissolution conditions were unable to discriminate between nanosized and coarse products, all products yielding very rapid dissolution. For the remaining compounds (cinnarizine, indomethacin, itraconazole, loviride, phenylbutazone, phenytoin), dissolution rates clearly increased due to nanosizing. Upon further analysis, it was found that compounds with more hydrophobic surfaces (cinnarizine, itraconazole, phenylbutazone) resulted in powders for which disintegration became a limiting factor for the overall dissolution process. These data strongly suggest that the need for inclusion of additional matrix formers is largely dictated by the surface properties of the drug compound for which the nanosuspension is made. Finally, for the three compounds that showed an important decrease of the dissolution rate (cinnarizine, itraconazole, phenylbutazone), four alternative matrix formers were evaluated: microcrystalline cellulose (Avicel®PH101), anhydrous dicalcium phosphate (Fujicalin®), colloidal silicon dioxide (Aerosil®200) and a hydrophobically modified inuline (Inutec®SP1) (Van Eerdenbrugh et al., submitted for publication-b). Concerning their ability with respect to preservation of rapid drug dissolution after spray-drying, the alternative matrix formers showed the following rank order: Fujicalin® < Avicel®PH101 < Aerosil®200 < Inutec®SP1, with Aerosil®200 and Inutec®SP1 showing very good results for all three drug compounds.

Studies in which further processing, such as pelletization and tabletting, are described are harder to find. Moreover, they frequently report on the formulation of dosage forms intended for controlled release purposes. The latter is a bit in contrast with the general appreciation of drug nanocrystals as being systems to achieve rapid dissolution. Although it can be expected that dissolution rate will decrease due to these processes, we could only find one patent describing the formulation of tablets (Naproxen) that still had very attractive characteristics with respect to rapid dissolution or onset of action. This is a nice illustration that it is possible, even after tabletting, to obtain a final dosage forms that, to a large extent, still exhibits the original characteristics of the parent nanosuspension, i.e. rapid dissolution.

Finally, it is interesting to briefly consider the inactive ingredients that can be found in the labeling information of the marketed products (Drugs@FDA). The following inactive ingredients can be found in the different marketed solid dosage forms: Rapamune® (sucrose, lactose, polyethylene glycol 8000, calcium sulfate, microcrystalline cellulose, pharmaceutical glaze, talc, titanium dioxide, magnesium stearate, povidone, poloxamer 188, polyethylene glycol 20,000, glyceryl monooleate, carnauba wax, and dl-alpha tocopherol), Emend® (sucrose, microcrystalline cellulose, hydroxypropyl cellulose and sodium lauryl sulfate), Tricor®: (hypromellose 2910 (3cps), docusate sodium, sucrose, sodium lauryl sulfate, lactose monohydrate, silicified microcrystalline cellulose, crospovidone, and magnesium stearate, polyvinyl alcohol, titanium dioxide, talc, soybean lecithin, xanthan gum) and Triglide® (crospovidone, lactose, monohydrate, mannitol, maltodextrin, carboxymethylcellulose sodium, egg lecithin, croscarmellose sodium, sodium lauryl sulfate, colloidal silicon dioxide, magnesium stearate, and monobasic sodium phosphate). It is remarkable that sucrose is used in the first three formulations and mannitol in the fourth. Although the functionality of inactive ingredients added remains speculative to some extent, these are the traditional matrix-formers.

5. Perspectives

Although drug nanocrystals are nowadays considered as a mature drug formulation strategy, a number of issues have still not received adequate attention in literature. Concerning nanosuspension stabilization, it is only during the last few years that reports attempting to rationalize the physicochemical aspects of the process using screening-based approaches have been published. The feasibility of miniaturizing of media milling for screening purposes, apart from its value during preclinical formulation evaluation, might be applied as a tool to further extend our understanding of nanosuspension stabilization. Concerning nanosuspension solidification, the majority of the current publications are dealing with freeze-drying or spray-drying technology. Processing steps as granulation, bead-layering and tabletting have been largely ignored until now. It is the authors' believe that further research aiming at deepening our understanding on nanosuspension stabilization as well as on the further downstream manufacturing processes can greatly contribute to future applications of drug nanocrystals for oral delivery purposes. There is, still, plenty of room at the bottom (Feynman, 1959).

References

Ain-Ai, A., Gupta, P.K., 2008. Effect of arginine hydrochloride and hydroxypropyl cellulose as stabilizers on the physical stability of high drug loading nanosuspensions of a poorly soluble compound. Int. J. Pharm. 351, 282–288.

Avantium. http://www.avantium.com/index.php.

British Standards Institution, 2005. PAS 71:2005—Vocabulary—Nanoparticles. http://www.bsi-global.com/upload/Standards%20&%20Publications/Nanotechnologies/PAS71.pdf.

Chen, H., Zhang, Z., McNulty, C., Olbert, C., Yoon, H.J., Lee, J.W., Kim, S.C., Seo, M.H., Oh, H.S., Lemmo, A.V., Ellis, S.J., Heimlich, K., 2003. A high-throughput combinatorial approach for the discovery of a cremophor EL-free paclitaxel formulation. Pharm. Res. 20, 1302–1308.

Chiang, P.C., Wahlstrom, J.L., Selbo, J.G., Zhou, S., Wene, S.P., Albin, L.A., Warren, C.J., Smith, M.E., Roberds, S.L., Ghosh, S., Zhang, L.L., Pretzer, D.K., 2007. 1,3-Dicyclohexyl urea nanosuspension for intravenous steady-state delivery in rats. J. Exp. Nanosci. 2, 239–250.

Choi, J.Y., Yoo, J.Y., Kwak, H.S., Nam, B.U., Lee, J., 2005. Role of polymeric stabilizers for drug nanocrystal dispersions. Curr. Appl. Phys. 5, 472–474.

Crisp, M.T., Tucker, C.J., Rogers, T.L., Williams, R.O., Johnston, K.P., 2007. Turbidimetric measurement and prediction of dissolution rates of poorly soluble drug nanocrystals. J. Control. Release 117, 351–359.

Cunningham, J., Merisko-Liversidge, E., Cooper, E.R., Liversidge, G.G., 15 July 2004. Milling microgram quantities of nanoparticulate candidate compounds. World Patent WO 2004/058216 A2.

Dai, W.G., Dong, L.C., Li, S., Pollock-Dove, C., Chen, J., Mansky, P., Eichenbaum, G., 2007. Parallel screening approach to identify solubility-enhancing formulations for improved bioavailability of a poorly water-soluble compound using milligram quantities of material. Int. J. Pharm. 336, 1–11.

Date, A.A., Patravale, V.B., 2004. Current strategies for engineering drug nanoparticles. Curr. Opin. Colloid Interface Sci. 9, 222–235.

Deng, Z., Xu, S., Li, S., 2008. Understanding a relaxation behavior in a nanoparticle suspension for drug delivery applications. Int. J. Pharm. 351, 236–243.

Derjaguin, B.V., Landau, L., 1941. Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes. Acta Phys. Chim. 14, 633–662.

Durán, J.D.G., Guindo, M.C., Delgado, A.V., Gonzáles-Caballero, F., 1995. Stability of monodisperse zinc sulfide colloidal dispersions. Langmuir 11, 3648–3655.

Elan. http://www.elan.com.

Fasano, A., 1998. Innovative strategies for the oral delivery of drugs and peptides. TIBTECH 16, 152–157.

FDA Orange Book. http://www.fda.gov/cder/ob/default.htm.

Drugs@FDA. http://www.accessdata.fda.gov/scripts/cder/drugsatfda/.

Feynman, R., 29 December 1959. There's plenty of room at the bottom (lecture). American Physical Society Meeting, Pasadena.

Gao, L., Zhang, D., Chen, M., Zheng, T., Wang, S., 2007. Preparation and characterization of an oridonin nanosuspension for solubility and dissolution velocity enhancement. Drug Dev. Ind. Pharm. 33, 1332–1339.

Gao, L., Zhang, D., Chen, M., Duan, C., Dai, W., Jia, L., Zhao, W., 2008. Studies on pharmacokinetics and tissue distribution of oridonin nanosuspensions. Int. J. Pharm. 355, 321–327.

Gardner, C.R., Almarsson, O., Chen, H., Morissette, S., Peterson, M., Zhang, Z., Wang, S., Lemmo, A., Gonzalez-Zugasti, J., Monagle, J., Marchionna, J., Ellis, S., McNulty, C., Johnson, A., Levinson, D., Cima, M., 2004a. Application of high throughput technologies to drug substance and drug product development. Comput. Chem. Eng. 28, 943–953.

Gardner, C.R., Walsh, C.T., Almarsson, O., 2004b. Drugs as materials: valuing physical form in drug discovery. Nat. Rev. Drug Discov. 3, 926–934.

Gonzáles-Caballero, F., de Dios Garciá López-Durán, J., 2000. Suspension formulation. In: Nielloud, F., Marti-Mestres, G. (Eds.), Pharmaceutical Emulsions and Suspensions, Drugs and the Pharmaceutical Sciences, vol. 105. Marcel Dekker, Inc., New York, pp. 127–190.

Grau, M.J., Kayser, O., Müller, R.H., 2000. Nanosuspensions of poorly soluble drugs—reproducibility of small scale production. Int. J. Pharm. 196, 155–159.

Gupta, R.B., 2006. Fundamentals of Drug Nanoparticles. In: Gupta, R.B., Kompella, U.B. (Eds.), Nanoparticle Technology for Drug Delivery, Drugs and the Pharmaceutical Sciences, vol. 159. Taylor & Francis Group, LLC, New York, pp. 1–19.

Hanafy, A., Spahn-Langguth, H., Vergnault, G., Grenier, P., Grozdanis, M.T., Lenhardt, T., Langguth, P., 2007. Pharmacokinetic evaluation of oral fenofibrate nanosuspensions and SLN in comparison to conventional suspensions of micronized drug. Adv. Drug Deliv. Rev. 59, 419–426.

Hansell, R.J., 3 February 2005. Laboratory scale milling process. United States Patent US 2005/0023386 A.

Hecq, J., Deleers, M., Fanara, D., Vranckx, H., Amighi, K., 2005. Preparation and characterization of nanocrystals for solubility and dissolution rate enhancement of nifedipine. Int. J. Pharm. 299, 167–177.

Hecq, J., Deleers, M., Fanara, D., Vranckx, H., Boulanger, P., Le Lamer, S., Amighi, K., 2006a. Preparation and in vitro/in vivo evaluation of nano-sized crystals for dissolution rate enhancement of ucb-35440-3, a highly dosed poorly water-soluble weak base. Eur. J. Pharm. Biopharm. 64, 360–368.

Hecq, J., Nollevaux, G., Deleers, M., Fanara, D., Vranckx, H., Peulen, O., Dandrifosse, G., Amighi, K., 2006b. Nifedipine nanocrystals: pharmacokinetic evaluation in the rat and permeability studies in Caco-2/HT29-5M21 (co)-cultures. J. Drug Del. Sci. Technol. 16, 437–442.

Hernández-Trejo, N., Kayser, O., Steckel, H., Müller, R.H., 2005. Characterization of nebulized buparvaquone nanosuspensions—effect of nebulization technology. J. Drug Target. 13, 499–507.

Horn, D., Rieger, J., 2001. Organic nanoparticles in the aqueous phase—theory, experiment, and use. Angew. Chem. Int. 40, 4331–4361.

Hu, J., Johnston, K.P., Williams, R.O., 2004. Nanoparticle engineering processes for enhancing the dissolution rates of poorly water soluble drugs. Drug Dev. Ind. Pharm. 30, 233–245.

Israelachvili, J., 1992. Intermolecular and Surface Forces, 2nd ed. Academic Press, London.

Jacobs, C., Kayser, O., Müller, R.H., 2000. Nanosuspensions as a new approach for the formulation for the poorly soluble drug tarazepide. Int. J. Pharm. 196, 161–164.

Jacobs, C., Kayser, O., Müller, R.H., 2001. Production and characterisation of mucoadhesive nanosuspensions for the formulation of bupravaquone. Int. J. Pharm. 214, 3–7.

Jacobs, C., Müller, R.H., 2002. Production and characterization of a budesonide nanosuspension for pulmonary administration. Pharm. Res. 19, 189–194.

Jahnke, S., 1998. The theory of high-pressure homogenization. In: Müller, R.H., Benita, S., Böhm, B.H.L. (Eds.), Emulsions and Nanosuspensions for the Formulation of Poorly Soluble Drugs. Medpharm Scientific, Stuttgart, pp. 177–200.

Jia, L., Wong, H., Cerna, C., Weitman, S.D., 2002. Effect of nanonization on absorption of 301029: ex vivo and in vivo pharmacokinetic correlations determined by liquid chromatography/mass spectrometry. Pharm. Res. 19, 1091–1096.

Jinno, J., Kamada, N., Miyake, M., Yamada, K., Mukai, T., Odomi, M., Toguchi, H., Liversidge, G.G., Higaki, K., Kimura, T., 2006. Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs. J. Control. Release 111, 56–64.

Joachim, C., 2005. To be nano or not to be nano? Nature Materials 4, 107–109.

Kassem, M.A., Rahman, A.A.A., Ghorab, M.M., Ahmed, M.B., Khalil, R.M., 2007. Nanosuspension as an ophthalmic delivery system for certain glucocorticoid drugs. Int. J. Pharm. 340, 126–133.

Kayser, O., Olbrich, C., Yardley, V., Kiderlen, A.F., Croft, S.L., 2003. Formulation of amphotericin B as nanosuspension for oral administration. Int. J. Pharm. 254, 73–75.

Keck, C.M., Müller, R.H., 2006. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenization. Eur. J. Pharm. Biopharm. 62, 3–16.

Kesisoglou, F., Panmai, S., Wu, Y., 2007a. Application of nanoparticles in oral delivery of immediate release formulations. Curr. Nanosci. 3, 183–190.

Kesisoglou, F., Panmai, S., Wu, Y., 2007b. Nanosizing—oral formulation development and biopharmaceutical evaluation. Adv. Drug Deliv. Rev. 59, 631–644.

Kipp, J.E., 2004. The role of solid nanoparticle technology in the parenteral delivery of poorly water-soluble drugs. Int. J. Pharm. 284, 109–122.

Kondo, N., Iwao, T., Masuda, H., Yamanouchi, K., Ishihara, Y., Yamada, N., Haga, T., Ogawa, Y., Yokoyama, K., 1993. Improved oral absorption of a poorly water-soluble drug, HO-221, by wet-bead milling producing particles in submicron region. Chem. Pharm. Bull. 41, 737–740.

Krause, K.P., Müller, R.H., 2001. Production and characterisation of highly concentrated nanosuspensions by high pressure homogenization. Int. J. Pharm. 214, 21–24.

Kumar, M.P., Rao, Y.M., Apte, S., 2007. Improved bioavailability of albendazole following oral administration of nanosuspension in rats. Curr. Nanosci. 3, 191–194.

Kumar, M.P., Rao, Y.M., Apte, S., 2008. Formulation of nanosuspensions of albendazole for oral administration. Curr. Nanosci. 4, 53–58.

Langguth, P., Hanafy, A., Frenzel, D., Grenier, P., Nhamias, A., Ohlig, T., Vergnault, G., Spahn-Langguth, H., 2005. Nanosuspension formulations for low-soluble drugs:

pharmacokinetic evaluation using spironolactone as model compound. Drug Dev. Ind. Pharm. 31, 319–329.
Lee, J., 2003. Drug nano- and microparticles processed into solid dosage forms: physical properties. J. Pharm. Sci. 92, 2057–2068.
Lee, J., Lee, S.J., Choi, J.Y., Yoo, J.Y., Ahn, C.H., 2005. Amphiphilic amino acid copolymers as stabilizers for the preparation of nanocrystal dispersion. Eur. J. Pharm. Sci. 24, 441–449.
Lee, J., Cheng, Y., 2006. Critical freezing rate in freeze drying nanocrystal dispersions. J. Control. Release 111, 185–192.
Lee, J., Choi, J.Y., Park, C.H., 2008. Characteristics of polymers enabling nano-comminution of water-insoluble drugs. Int. J. Pharm. 355, 328–336.
Lipinski, C., 2002. Poor aqueous solubility—an industry wide problem in drug discovery. Am. Pharm. Rev. 5, 82–85.
Liversidge, G.G., Phillips, C.P., Cundy, K.C., 12 April 1994. Method to reduce particle size growth during lyophilization. US Patent US 5,302,401 A.
Liversidge, G.G., Conzentino, P., 1995. Drug particle size reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats. Int. J. Pharm. 125, 309–313.
Liversidge, G.G., Cundy, K.C., 1995. Particle size reduction for improvement of oral bioavailability of hydrophobic drugs. I. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. Int. J. Pharm. 125, 91–97.
Liversidge, G.G., Eickhoff, W.M., Ruddy, S.B., Mueller, K.R., Roberts, M.E., Engers, D.A., 20 August 1998. Formulations of nanoparticle naproxen tablets. World Patent WO 98/35666.
Mansky, P., Dai, W.G., Li, S., Pollock-Dove, C., Daehne, K., Dong, L., Eichenbaum, G., 2007. Screening method to identify preclinical liquid and semi-solid formulations for low solubility compounds: miniaturization and automation of solvent casting and dissolution testing. J. Pharm. Sci. 96, 1548–1563.
Marti-Mestres, G., Nielloud, F., 2000. Main surfactants used in the pharmaceutical field. In: Nielloud, F., Marti-Mestres, G. (Eds.), Pharmaceutical Emulsions and Suspensions, Drugs and the Pharmaceutical Sciences, vol. 105. Marcel Dekker, Inc., New York, pp. 1–18.
Merisko-Liversidge, E., Sarpotdar, P., Bruno, J., Hajj, S., Wei, L., Peltier, N., Rake, J., Shaw, J.M., Pugh, S., Polin, L., Jones, J., Corbett, T., Cooper, E., Liversidge, G.G., 1996. Formulation and antitumor activity evaluation of nanocrystalline suspensions of poorly soluble anticancer drugs. Pharm. Res. 13, 272–278.
Merisko-Liversidge, E., Linden, W., 27 March 2003. Stabilization of active agents by formulation into nanoparticulate form. World Patent WO 03/024424 A1.
Merisko-Liversidge, E., Liversidge, G.G., Cooper, E.R., 2003. Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur. J. Pharm. Sci. 18, 113–120.
Merisko-Liversidge, E., McGurk, S.L., Liversidge, G.G., 2004. Insulin nanoparticles: a novel formulation approach for poorly water soluble Zn-insulin. Pharm. Res. 21, 1545–1553.
Möschwitzer, J., Achleitner, G., Pomper, H., Müller, R.H., 2004. Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology. Eur. J. Pharm. Biopharm. 58, 615–619.
Möschwitzer, J., Müller, R.H., 2006. Spray coated pellets as carrier system for mucoadhesive drug nanocrystals. Eur. J. Pharm. Biopharm. 62, 282–287.
Mouton, J.W., van Peer, A., de Beule, K., Van Vliet, A., Donnelly, J.P., Soons, P.A., 2006. Pharmacokinetics of itraconazole and hydroxyitraconazole in healthy subjects after single and multiple doses of a novel formulation. Antimicrob. Agents Chemother. 50, 4096–4102.
Müller, R.H., Böhm, B.H.L., 1998. Nanosuspensions. In: Müller, R.H., Benita, S., Böhm, B.H.L. (Eds.), Emulsions and Nanosuspensions for the Formulation of Poorly Soluble Drugs. Medpharm Scientific, Stuttgart, pp. 149–174.
Müller, R.H., Peters, K., 1998. Nanosuspensions for the formulation of poorly soluble drugs. I. Preparation by a size-reduction technique. Int. J. Pharm. 2, 229–237.
Müller, R.H., Böhm, B.H.L., Grau, M.J., 1999a. Nanosuspensions – formulations for poorly soluble drugs with poor bioavailability – 1st communication: production and properties. Pharm. Ind. 61, 74–78.
Müller, R.H., Böhm, B.H.L., Grau, M.J., 1999b. Nanosuspensions – formulations for poorly soluble drugs with poor bioavailability – 2nd communication: stability, biopharmaceutical aspects, possible drug forms and registration aspects. Pharm. Ind. 61, 175–178.
Müller, R.H., Böhm, B.H.L., Grau, M.J., 2000a. Nanosuspensions: a formulation approach for poorly soluble and poorly bioavailable drug. In: Wise, D.L. (Ed.), Handbook of Pharmaceutical Controlled Release Technology. Marcel Dekker, Inc., New York, pp. 345–357.
Müller, R.H., Jacobs, C., Kayser, O., 2000b. Nanosuspensions for the formulation of poorly soluble drugs. In: Nielloud, F., Marti-Mestres, G. (Eds.), Pharmaceutical Emulsions and Suspensions, Drugs and the Pharmaceutical Sciences, vol. 105. Marcel Dekker, Inc., New York, pp. 383–407.
Müller, R.H., Jacobs, C., Kayser, O., 2001. Nanosuspensions as particulate drug formulations in therapy—rationale for development and what we can expect for the future. Adv. Drug Deliv. Rev. 47, 3–19.
Müller, R.H., Jacobs, C., 2002. Buparvaquone mucoadhesive nanosuspension: preparation, optimisation and long-term stability. Int. J. Pharm. 237, 151–161.
Müller, R.H., Kayser, O., Jacobs, J., 2003. DissoCubes—a novel formulation for poorly soluble and poorly bioavailable drugs. In: Rathborn, M.J., Hadgraft, J., Roberts, M.S. (Eds.), Modified-Release Drug Delivery Technology. Drugs and the Pharmaceutical Sciences, vol. 126. Marcel Dekker, Inc., New York, pp. 135–149.
Müller, R.H., Keck, C.M., 2004. Challenges and solutions for the delivery of biotech drugs—a review of drug nanocrystal technology and lipid nanoparticles. J. Biotechnol. 113, 151–170.
Müller, R.H., Möschwitzer, J., Bushrab, F.N., 2006. Manufacturing of nanoparticles by milling and homogenization techniques. In: Gupta, R.B., Kompella, U.B. (Eds.), Nanoparticle Technology for Drug Delivery, Drugs and the Pharmaceutical Sciences, vol. 159. Taylor & Francis Group, LLC, New York, pp. 21–51.
Na, G.C., Stevens, H.J., Yuan, B.O., Rajagopalan, N., 1999. Physical Stability of Ethyl Diatrizoate Nanocrystalline Suspension in Steam Sterilization. Pharm. Res. 16, 569–574.
Ostrander, K.D., Bosch, H.W., Bondanza, D.M., 1999. An in-vitro assessment of a NanoCrystal™ beclomethasone dipropionate colloidal dispersion via ultrasonic nebulization. Eur. J. Pharm. Biopharm. 48, 207–215.
Patravale, V.B., Date, A.A., Kulkarni, R.M., 2004. Nanosuspensions: a promising drug delivery strategy. J. Pharm. Pharmacol. 56, 827–840.
Peters, K., Müller, R.H., Craig, D.Q.M., 1999. An investigation into the distribution of lecithins in nanosuspension systems using low frequency dielectric spectroscopy. Int. J. Pharm. 184, 53–61.
Peters, K., Leitzke, S., Diederichs, J.E., Borner, K., Hahn, H., Müller, R.H., Ehlers, S., 2000. Preparation of a clofazimine nanosuspension for intravenous use and evaluation of its therapeutic efficacy in murine *Mycobacterium avium* infection. J. Antimicrob. Chemother. 45, 77–83.
Rabinow, B.E., 2004. Nanosuspensions in drug delivery. Nat. Rev. Drug Discov. 3, 785–796.
Rabinow, B., Chaubal, M.V., 2006. Injectable nanoparticles for efficient drug delivery. In: Gupta, R.B., Kompella, U.B. (Eds.), Nanoparticle Technology for Drug Delivery, Drugs and the Pharmaceutical Sciences, vol. 159. Taylor & Francis Group, LLC, New York, pp. 199–229.
Rabinow, B., Kipp, J., Papadopoulos, P., Wong, J., Glosson, J., Gass, J., Sun, C.S., Wielgos, T., White, R., Cook, C., Barker, K., Wood, K., 2007. Itraconazole IV nanosuspension enhances efficacy through altered pharmacokinetics in the rat. Int. J. Pharm. 339, 251–260.
Rao, G.C.S., Kumar, M.S., Mathivanan, N., Rao, M.E.B., 2004. Nanosuspensions as the most promising approach in nanoparticulate drug delivery systems. Pharmazie 59, 5–9.
Rowe, R.C., Sheskey, R.J., Weller, P.J., 2003. Handbook of Pharmaceutical Excipients, 4th ed. Pharmaceutical Press, London.
Royal Society and Royal Academy of Engineering, 2004. Nanoscience, and nanotechnology: opportunities and uncertainties. http://www.nanotec.org.uk/finalReport.htm.
Schöler, N., Krause, K., Kayser, O., Müller, R.H., Borner, K., Hahn, H., Liesenfeld, O., 2001. Atovaquone nanosuspensions show excellent therapeutic effect in a new murine model of reactivated toxoplasmosis. Antimicrob. Agents Chemother. 45, 1771–1779.
Shanbhag, A., Rabel, S., Nauka, E., Casadevall, G., Shivanand, P., Eichenbaum, G., Mansky, P., 2008. Method for screening of solid dispersion formulations of low-solubility compounds—miniaturization and automation of solvent casting and dissolution testing. Int. J. Pharm. 351, 209–218.
Sigfridsson, K., Forssén, S., Holländer, P., Skantze, U., de Verdier, J., 2007. A formulation comparison, using a solution and different nanosuspensions of a poorly soluble compound. Eur. J. Pharm. Biopharm. 67, 540–547.
SkyePharma. http://skyepharma.com.
Symyx. http://www.symyx.com/index.php.
TransForm Pharmaceuticals. http://www.transformpharma.com/.
Van Eerdenbrugh, B., Froyen, L., Martens, J.A., Blaton, N., Augustijns, P., Brewster, M., Van den Mooter, G., 2007. Characterization of physico-chemical properties and pharmaceutical performance of sucrose co-freeze-dried solid nanoparticulate powders of the anti-HIV agent loviride prepared by media milling. Int. J. Pharm. 338, 198–206.
Van Eerdenbrugh, B., Vermant, J., Martens, J.A., Froyen, L., Van Humbeeck, J., Augustijns, P., Van den Mooter, G., submitted for publication-a. A Screening Study of Surface Stabilization in the Production of Drug Nanocrystals. J. Pharm. Sci.
Van Eerdenbrugh, B., Froyen, L., Van Humbeeck, J., Martens, J.A., Augustijns, P., Van den Mooter, G., submitted for publication-b. Alternative matrix formers for nanosuspension solidification: dissolution performance and X-ray microanalysis as an evaluation tool for powder dispersion. Eur. J. Pharm. Sci.
Van Eerdenbrugh, B., Froyen, L., Van Humbeeck, J., Martens, J.A., Augustijns, P., Van den Mooter, G., submitted for publication-c. Scaling down of drug nanosuspension production: process aspects and physico-chemical characterization. AAPS PharmSciTech.
Van Eerdenbrugh, B., Froyen, L., Van Humbeeck, J., Martens, J.A., Augustijns, P., Van den Mooter, G., 2008a. Drying of crystalline drug nanosuspensions—the importance of surface hydrophobicity on dissolution behavior upon redispersion. Eur. J. Pharm. Sci. 35, 127–135.
Van Eerdenbrugh, B., Vercruysse, S., Martens, J.A., Vermant, J., Froyen, L., Van Humbeeck, G., Van den Mooter, L., Augustijns, P., 2008b. Microcrystalline cellulose, a useful alternative for sucrose as a matrix former during freeze-drying of drug nanosuspensions—a case study with itraconazole. Eur. J. Pharm. Biopharm. 70, 590–596.
van Oss, C.J., Giese, R.F., Costanzo, P.M., 1990. DLVO and non-DLVO interactions in hectorite. Clays Clay Miner. 38, 151–159.
van Oss, C.J., 1994. Interfacial Forces in Aqueous Media. Marcel Dekker, New York.
Vergote, G.J., Vervaet, C., Van Driessche, I., Hoste, S., De Smedt, S., Demeester, J., Jain, R.A., Ruddy, S., Remon, J.P., 2001. An oral controlled release matrix pellet formulation containing nanocrystalline ketoprofen. Int. J. Pharm. 219, 81–87.
Vergote, G.J., Vervaet, C., Van Driessche, I., Hoste, S., De Smedt, S., Demeester, J., Jain, R.A., Ruddy, S., Remon, J.P., 2002. In vivo evaluation of matrix pellets containing nanocrystalline ketoprofen. Int. J. Pharm. 240, 79–84.

Verwey, E.J.W., Overbeek, J.Th.G., 1948. Theory of the Stability of Lyophobic Colloids. Elsevier, Amsterdam.
Wahlstrom, J.L., Chiang, P.C., Ghosh, S., Warren, C.J., Wene, S.P., Albin, L.A., Smith, M.E., Roberds, S.L., 2007. Pharmacokinetic evaluation of a 1,3-dicyclohexylurea nanosuspension formulation to support early efficacy assessment. Nanoscale Res. Lett. 2, 291–296.
Wiedmann, T.S., DeCastro, L., Wood, R.W., 1997. Nebulization of NanoCrystals™: production of a respirable solid-in-liquid-in-air colloidal dispersion. Pharm. Res. 14, 112–116.
Wong, J., Brugger, A., Khare, A., Chaubal, M., Papadopoulos, P., Rabinow, B., Kipp, J., Ning, J., 2008. Suspensions for intravenous (IV) injection: a review of development, preclinical and clinical aspects. Adv. Drug Deliv. Rev. 60, 939–954.
Wu, Y., Loper, A., Landis, E., Hettrick, L., Novak, L., Lynn, K., Chen, C., Thompson, K., Higgins, R., Batra, U., Shelukar, S., Kwei, G., Storey, D., 2004. The role of biopharmaceutics in the development of a clinical nanoparticle formulation of MK-0869: a Beagle dog model predicts improved bioavailability and diminished food effect on absorption in human. Int. J. Pharm. 285, 135–146.
Xiong, R., Lu, W., Li, J., Wang, P., Xu, R., Chen, T., 2008. Preparation and characterization of intravenously injectable nimodipine nanosuspension. Int. J. Pharm. 350, 338–343.
Zhang, D., Tan, T., Gao, L., Zhao, W., Wang, P., 2007. Preparation of azithromycin nanosuspensions by high pressure homogenization and its physicochemical characteristics studies. Drug Dev. Ind. Pharm. 33, 569–575.
Zheng, J.Y., Bosch, H.W., 1997. Sterile filtration of NanoCrystal™ drug formulations. Drug Dev. Ind. Pharm. 23, 1087–1093.