International Journal of Pharmaceutics 396 (2010) 210–218

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Miconazole nanosuspensions: Influence of formulation variables on particle size reduction and physical stability

Ana M. Cerdeira[a], Marco Mazzotti[b], Bruno Gander[c,*]

[a] Cilag AG, Hochstrasse 201, 8205 Schaffhausen, Switzerland
[b] Institute of Process Engineering, ETH Zurich, Sonneggstrasse 3, 8092 Zurich, Switzerland
[c] Institute of Pharmaceutical Sciences, ETH Zurich, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland

ARTICLE INFO

Article history:
Received 16 March 2010
Received in revised form 8 June 2010
Accepted 14 June 2010
Available online 19 June 2010

Keywords:
Nanosuspension
Nanogrinding
Nanonization
Suspension stability
Miconazole
Drug solubility

ABSTRACT

New drug substances from early development are often poorly water-soluble, which causes poor bioavailability upon peroral administration and hampers drug administration through other routes such as the parenteral or ocular routes. One approach to improve drug solubility and administration flexibility is by wet milling to nanosize. Particle size reduction increases the surface energy which requires adequate stabilization by excipients. In this study, the practically water-insoluble miconazole was nanoground, and a variety of surface active and polymeric excipients were tested for their stabilizing effects. For efficient milling, two preformulation criteria had to be fulfilled: a relatively low contact angle (<70°) and high dispersibility of the native drug particles in the milling medium. Hydroxypropylcellulose (HPC-LF) in combination with sodium dodecyl sulfate (SDS) stabilized best the miconazole nanosuspensions. A design of experiments was used to achieve drug particle mean sizes of 140–170 nm by varying the concentrations of miconazole (5 and 20%, w/w), SDS (0.05 and 0.2%, w/w), and HPC-LF (1.25 and 5%, w/w). Further experiments revealed that minimal 0.0125% SDS and 3.125% HPC-LF were required for miconazole nanogrinding and nanosuspension stabilisation. Storage of the nanosuspensions at 5 °C for up to 6 months caused only minor changes, whereas storage at 25 °C resulted in particle agglomeration and single crystal growth. Altogether the study showed that excellent wetting of drug particles as well as their electrostatic and steric stabilization by excipients is necessary to produce stable nanosuspensions by nanogrinding.

1. Introduction

Many new drug substances are only very slightly soluble or even practically insoluble. A substantial portion (40%) of these drugs fails full development, because of their poor and highly variable bioavailability (Gardner et al., 2004; Riley, 2006). Upon peroral administration, very slightly water-soluble or practically water-insoluble drugs have a limited and variable or erratic oral absorption (Crison, 2000). Further, the low solubility of such drugs limits their parenteral use. Low solubility of drug substances may result from hydrophobicity or high lattice energy. Highly hydrophobic drug substances possess insufficient capacity of molecular interactions with water, whereas molecules with high lattice energy resist to the weakening of the lattice upon molecular interactions with water (Kipp, 2004). According to the law of Noyes–Whitney, low solubility yields a low concentration gradient towards the bulk of the solution and, thereby, a low dissolution rate. Therefore, absorption and bioavailability of perorally administered drugs that possess good permeability, but low solubility, can be improved by increasing either the solubility or the surface area of the drug substance, both resulting in increased dissolution rates. A very common way to increase drug substance surface area is by micronization, which produces particles in the size range of 2–5 μm. However, when the solubility of a drug is very low, i.e., below approximately 1 mg/ml, micronization is generally insufficient to increase adequately the drug dissolution rate and absorption in the gastro-intestinal tract (Muller et al., 2001).

Nanonization has become a popular approach to produce particles in the size range of 200–400 nm, to improve both the dissolution rate and the solubility of the compound (Liversidge et al., 1992). The latter phenomenon is due to the well-known dependency of solubility on particle size as described by the Ostwald–Freundlich equation. Breakage of micron-sized drug crystals into nanoparticles creates an increased particle surface area, which is thermodynamically unfavourable. Thus, nanosized particles tend to agglomerate to reduce their surface area. Particle agglomeration in nanosuspensions can be prevented by steric and electrostatic stabilization using polymeric and/or surfactant excip-

* Corresponding author. Tel.: +41 44 6337312; fax: +41 44 6331314.
E-mail address: bruno.gander@pharma.ethz.ch (B. Gander).

0378-5173/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.06.020

Table 1
Miconazole suspension formulations used for screening polymeric and surfactant stabilizers.

Miconazole concentration[a] (%, w/w)	Surfactant type and concentration[a] (%, w/w)	Polymer type and concentration[a] (%, w/w)
5	SDS, 0.05	HPC-EF, 1.25 HPMC, 1.25 PVP, 1.25 Poloxamer, 1.25 (without SDS)
10	SDS, 0.10	HPC-EF, 2.5 HPC-LF, 2.5 HPMC, 2.5 PVP, 2.5 Poloxamer, 2.5 (without SDS)
10	No surfactant SDS, 0.1 SD, 0.1 BK, 0.1	HPC-LF, 2.5

[a] Single line information in each row applies to all variables of this row.

ients (Rabinow, 2004). Most nanosuspensions are thus composed of an aqueous medium (e.g., purified water), a nanosuspended drug substance of maximal 400 mg/ml, and adequate excipients for nanogrinding and particle stabilization (Merisko-Liversidge et al., 2003). Both the type and concentration of excipient(s) are important for particle size reduction and physical stabilization of the formulations. Physically stable nanosuspensions are obtained at drug substance-to-excipient ratios of 20:1–2:1 (Merisko-Liversidge et al., 2003). Therefore, inadequate types or amounts of excipients may either cause particle agglomeration due to the high surface energy of the nanoparticles or crystal growth due to the drug substance solubility increase. Electrostatic and steric mechanisms are mediated by combining ionic surfactants and polymers (Rabinow, 2004). Most commonly used polymeric excipients for nanosuspensions include cellulose ethers (e.g., hydroxypropylcellulose, hydroxypropylmethylcellulose), povidone, and poloxamers (Liversidge et al., 1992; Merisko-Liversidge et al., 2003; Kesisoglou et al., 2007). The surfactant excipients can be non-ionic, such as polysorbate (Tween 80), or anionic, such as sodium dodecyl sulfate or sodium docusate. Cationic surfactants are less frequently used (Kesisoglou et al., 2007).

Physical stabilization of nanosuspensions is a major challenge. Alterations in particle size distribution, polymorphic and solvate forms need to be carefully analyzed and monitored during storage (Kipp, 2004). Nanosuspension stability depends on (i) the solid state properties of the nanoparticles (density, hardness, number and type of lattice defects), (ii) the interfacial properties (wetting and interfacial energy between nanoparticles and medium, structure of the solid-liquid interface), and (iii) the properties of the suspending medium (viscosity, drug solubility, presence of micelles and their interaction with the dissolved and solid drug). Instability of nanosuspensions may manifest by a shift of particle size distribution to larger sizes, irreversible agglomeration, or solid phase transformation (Kipp, 2004).

Despite the numerous challenges, nanosuspensions represent a very promising, rather general formulation approach to increase solubility and dissolution rate of very slightly soluble or practically water-insoluble solid drug substances. Furthermore, nanosuspensions are suitable for administration by various routes (parenteral, oral, ophthalmic and nasal), which is a eminent advantage over other dosage forms. Although numerous studies have explored drug substance nanogrinding and nanosuspensions, the parameters affecting nanogrinding and particle stabilization during storage are still not well understood (Augustijns et al., 2008). The aim of this study was, therefore, to evaluate the importance of the concentration of miconazole drug substance and of the type and concentration of surfactant and polymeric excipients on the physical characteristics of miconazole nanosuspensions during milling and storage. Miconazole was selected as it is practically insoluble in water, but possesses high permeability, which makes it an excellent candidate for nanogrinding. Miconazole is a well-known imidazole, used as base or nitrate salt, for treatment of superficial candidiasis, dermatophytosis, and pityriasis versicolor (Sweetman, 2006).

2. Materials and methods

2.1. Materials

Miconazole (lot # R018134PUC701, Janssen Pharmaceutica, Geel, Belgium) used for nanogrinding had a volume-based mean diameter of d_{50} = 27 μm, and 10% and 90% undersize percentiles of d_{10} = 14 and d_{90} = 49 μm, respectively. Sodium dodecyl sulfate [SDS] (Texapon® K12P, Cognis, Düsseldorf, Germany), sodium docusate [SD] (Cytec Industries, Belmont West Virginia, USA), benzalkonium chloride [BK] (Sigma–Aldrich, Schnelldorf, Germany), hydroxypropylcellulose [HPC-LF, HPC-EF] (Klucel® LF, Klucel® EF, Hercules, Doel, Belgium), povidone [PVP] (Plasdone® K29/32, ISP Technologies, Texas City, US), poloxamer [poloxamer] (Pluronic® F68, BASF, Ludwigshafen, Germany), hydroxypropylmethylcellulose [HPMC] (Hypromellose 2910, Methocel® E15 LV, Colorcon, Dow Chemicals, Dartford, UK), and were all used as received.

2.2. Production of nanosuspensions

For nanogrinding miconazole, solutions of surfactant and polymer stabilizers in purified water were first prepared. Miconazole (d_{10} = 14 μm; d_{50} = 27 μm; d_{90} = 49 μm) was then dispersed in the stabilizer solution. Initial experiments were designed to screen most suitable surfactant and polymer stabilizers (Table 1). Nanogrinding was performed in a high-energy mill (LabStar, Netzsch, Selb, Germany) filled (to 83%, w/v) with yttrium-stabilized zirconium oxide beads (0.8 mm in diameter). Nanogrinding was performed in circulation mode using 300 g of suspension, a pump-speed of 41 rpm, and a stirrer-tip-speed of 3400 rpm (10 m/s); the duration of the process was up to 60 min.

Table 2
Formulation factors and levels according to a 2^3 experimental design.

Level	Miconazole (%, w/w)	SDS (%, w/w)	HPC-LF (%, w/w)
(+1)	20	0.2	5
(−1)	5	0.05	1.25
(0)	12.5	0.125	3.125

2.3. Design of experiments (DOE)

A 2^3 DOE (Table 2) was used to study the effects and interactions of miconazole, SDS, and HPC-LF on particle size distribution. A centre point with replicate was introduced in the design to estimate the curvature and the pure error. The data were fitted according to the following polynomial equation:

$$Y = a_0 + a_iX_i + a_jX_j + a_kX_k + a_{ij}X_iX_j + a_{ik}X_iX_k + a_{jk}X_jX_k + a_{ijk}X_iX_jX_k \quad (1)$$

where a_0 is the overall mean response (mean particle size), a_i, a_j, and a_k are the main effect coefficients, a_{ij}, a_{ik}, a_{jk}, and a_{ijk} are the coefficients of the interaction effects (first and second order), and X_i, X_j and X_k are the factors (miconazole, SDS, HPC-LF). The statistical design and evaluation of the obtained experimental data was carried out with the software Minitab® 15 (Minitab Inc.). The model was reduced by removing non-significant coefficients ($\alpha = 0.05$). The significance and validity of the model was estimated by analysis of variance (ANOVA). Additional experiments were performed to explore the corners of the DOE and optimize the formulations.

2.4. Particle size distribution by laser light diffraction

Particle size distribution (volume based) was measured by laser light diffraction (Mastersizer 2000, Malvern Instruments, Worcestershire, UK), using the small volume dispersion unit (Hydro 2000 Micro Precision). The Mie theory (dispersant refractive index = 1.33; real particle refractive index = 1.55; imaginary part of the particle refractive index = 0.001) was used for particle size calculation. The nanosuspensions were diluted with purified water to obtain an appropriate obscuration. Particle sizes were expressed by the volume-based 50% (d_{50}) and 90% (d_{90}) diameter percentiles.

2.5. Optical microscopy

Optical microscopic pictures of miconazole suspensions in different stabilizer solutions were taken before milling (Zeiss Axiophot, Zürich, Switzerland).

2.6. Determination of contact angle

The dynamic contact angle between miconazole powder compacts and water was measured by the sessile drop method (Krüss DSA100, Hamburg, Germany and software Krüss drop shape analysis DSA1, Hamburg, Germany). The static contact angle between miconazole and stabilizer solutions was measured in triplicates using the powder method. The contact angle was calculated using the Washburn equation (Aulton, 2007).

2.7. Quantification of miconazole solubility in the nanosuspensions

Miconazole nanosuspensions were centrifuged (ultracentrifuge Sorvall, Thermo Fisher Scientific, Waltham, USA) at 50,000 rpm (minimum of 3 h), and the supernatant assayed for drug content by HPLC. Centrifugation was preferred over ultrafiltration, because of the difficulty experienced with filtering some of the relatively viscous (Fig. 5) suspensions, which caused filter clogging. Miconazole was assayed by a validated method using reversed phase HPLC with UV detection at 230 nm (Waters Alliance HPLC system, Milford, MA, USA) and a C18 column (Zorbax®, 10 cm length, 4.6 mm ID, 3.5 μm particle size). The drug was eluted with 10 mM di-sodium hydrogen phosphate of pH 7.5 (solvent A) and acetonitrile (solvent B) according to the gradient reported in Table 3. Linearity was confirmed between 0.5 and 700 μg/ml, and the accuracy was ±30% for 0.5–1 μg/ml, ±20% for 1–10 μg/ml; ±10% for 10–200 μg/ml, and ±3% for 200–700 μg/ml.

Table 3
HPLC solvent gradient sequence for assaying miconazole.

	Time (min)			
	0	30	35	40
Phase				
A (%, v/v)	80	35	80	80
B (%, v/v)	20	65	20	20

2.8. Viscosity measurement of the nanosuspensions

The viscosity of the nanosuspensions was determined using a rheometer (Rheostress® RS600 Haake, ThermoScientific, Waltham, USA). The measurements were performed at 20 ± 0.1 °C using rotational mode (constant shear rate of 100 s^{-1}) and cone-plate geometry (plate diameter 60 mm, cone angle 1°). To avoid shear history effects, samples were kept at rest for 5 min after their application on the sensor system.

2.9. Zeta-potential measurement of the nanosuspensions

Zeta-potential was measured using a Zetasizer (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK). The samples were adequately diluted with deionised water and placed in an electrophoretic cell. The mean zeta-potential was calculated from the electrophoretic mobility using the Smoluchowski equation (Aulton, 2007).

2.10. Particle size stability during storage

Nanosuspensions were stored in glass bottles (type I glass) with polypropylene caps, at 5 °C and 25 °C/60% RH for a period of up to 6 months. The stability was assessed in terms of particle size distributions (at 0 and 6 months), and drug solubility and zeta-potential (at 0 and 3 months).

3. Results

3.1. Excipient screening for miconazole nanogrinding

To select a suitable polymeric excipient, miconazole (5 and 10%, w/w) was nanoground using SDS (0.05 and 0.1%, w/w) and different types of polymers (1.25 and 2.5%, w/w) (Table 1; Figs. 1 and 2). The higher concentrations of miconazole and excipients promoted particle size reduction (Fig. 1 *versus* Fig. 2). The polymeric sta-

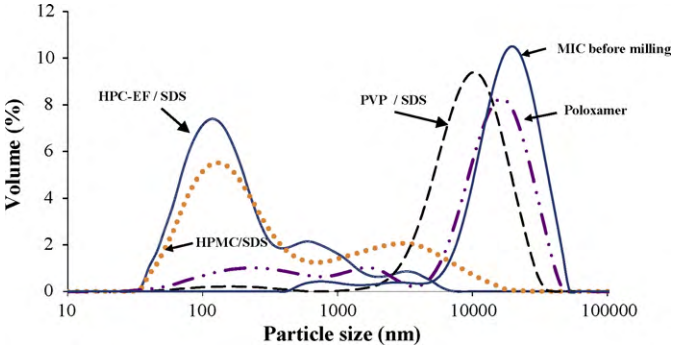

Fig. 1. Effect of the type of polymeric excipient on the efficiency of miconazole nanogrinding in terms of volume distributions of particle sizes. The formulations contained 5% (w/w) miconazole, 1.25% (w/w) polymer, and 0.05% (w/w) SDS. SDS was not present when poloxamer was used as polymeric stabilizer.

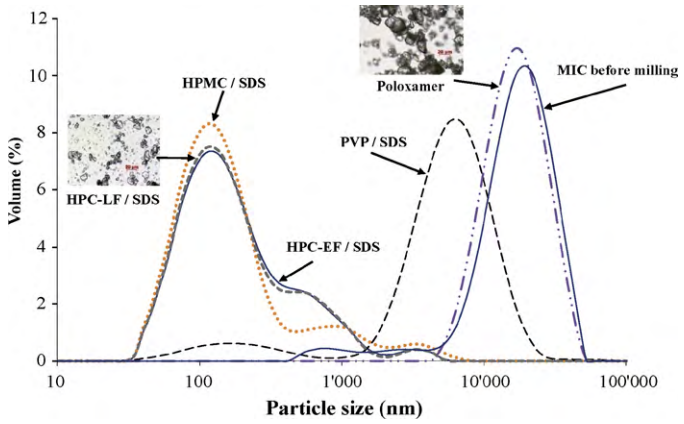

Fig. 2. Effect of the type of polymeric excipient on the efficiency of miconazole nanogrinding in terms of volume distributions of particle sizes. Micrographs (insets) illustrate two extreme cases of dispersibility of the native drug particles in the aqueous medium before nanogrinding. The formulations contained 10% (w/w) miconazole, 2.5% (w/w) polymer, and 0.1% (w/w) SDS. SDS was not present when poloxamer was used as polymeric stabilizer.

bilizers HPMC and HPC were found to be highly effective for nanogrinding miconazole, whereas poloxamer (non-ionic polymeric surfactant used without SDS) and PVP/SDS were ineffective (Figs. 1 and 2). The reasons for the inefficiency of poloxamer and PVP/SDS remain unknown. Nonetheless, microscopic observation of unground miconazole dispersions revealed large aggregates in poloxamer and PVP/SDS solutions, but well dispersed particles with the other excipients, as illustrated exemplarily for the poloxamer and HPC-LF/SDS formulations in Fig. 2.

To select a suitable surfactant, miconazole (10%, w/w) was milled using HPC-LF (2.5%, w/w) and different types of surfactants (0.1%, w/w) (Table 1, Fig. 3), because HPC-LF was found in the previous experiment to be a highly effective polymeric excipient for nanogrinding of miconazole. While all surfactants facilitated nanogrinding and yielded nanoparticles with similar d_{50} values (d_{50} of 155–175 nm) (Fig. 3, inset), SDS was the most effective in minimizing the large particle size fraction (2–10 μm) of the bimodal size distribution (Fig. 3). Using SDS, the particle size fraction of 2–10 μm represented less than 1% (volume) of the entire particle population. Here again, the extent of particle size reduction seemed to be predetermined by the degree of dispersion of unground miconazole in the different stabilizer solutions, as observed microscopically and illustrated exemplarily for the HPC-LF/SD and HPC-LF/SDS formulations in Fig. 3.

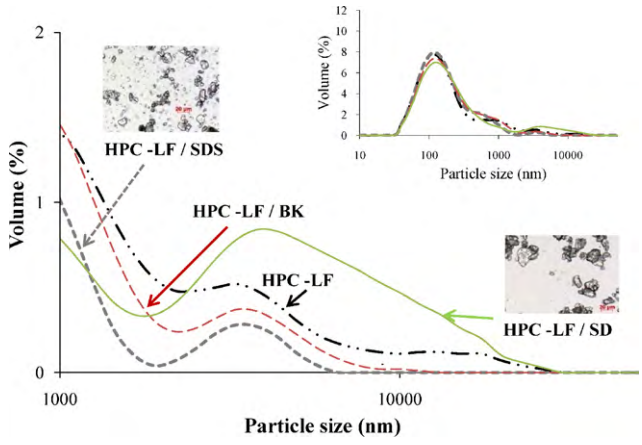

Fig. 3. Effect of the type of surfactant on the efficiency of miconazole nanogrinding in terms of volume distributions of particle sizes. Micrographs (insets) illustrate two extreme cases of dispersibility of the native drug particles in the aqueous medium before nanogrinding. The formulations contained 10% (w/w) miconazole, 2.5% (w/w) HPC-LF, and 0.1% (w/w) surfactant. One batch was prepared without surfactant.

Table 4
Solubility and contact angle of miconazole in stabilizer solutions before nanogrinding.

Excipient	Miconazole solubility (μg/ml)	Contact angle mean ± SD (°)
Surfactant: 0.1% (w/w); HPC-LF: 2.5% (w/w)		
None	3	86 ± 1
SD	4	46 ± 1
BK	7	56 ± 3
SDS	95	43 ± 3
Polymer: 2.5% (w/w); SDS: 0.1% (w/w)		
Poloxamer (without SDS)	0.5	89 ± 0
PVP	61	89 ± 0
HPMC	93	65 ± 1
HPC-LF	95	43 ± 3
HPC-EF	102	53 ± 4

A key requisite for nanogrinding is adequate particle wetting. While the contact angle between miconazole and pure water was above 140°, this value was reduced to 86° by addition of 2.5% HPC-LF to the aqueous medium (Table 4). Wetting was mostly enhanced by the use of surfactants (0.1%, w/w). Solutions of SD, BK and SDS all reduced the contact angle to values in the range of 43–56° (Table 4). Similar contact angles were observed between miconazole and the solutions containing SDS (0.1%, w/w) in combination with either HPMC, or HPC-EF, or HPC-LF (2.5%, w/w). Surprisingly, solutions of both PVP/SDS and poloxamer presented a rather high contact angle with miconazole, which was similar to that of HPC-LF alone. The poor wetting of miconazole by PVP/SDS and poloxamer coincides with the inefficient nanogrinding in the presence of these excipients (Figs. 1 and 2).

Surface active excipients not only provide particle wetting, but can also form micelles and, thereby, solubilise water-insoluble compounds. This might be critical in nanosuspensions, because of potential Ostwald ripening during storage (Merisko-Liversidge et al., 2003). Therefore, the effect of the different excipients on miconazole solubility was assessed (Table 4). In pure water and in HPC-LF solutions without surfactant, miconazole solubility was very low (3 μg/ml), and it was increased only slightly by the addition of BK and SD. In contrast, SDS increased substantially the miconazole solubility, i.e., to 95 μg/ml. In agreement with the observations of wetting, addition of PVP to the SDS solution lowered the interaction with the drug, thereby lowering the solubility (61 μg/ml). Poloxamer alone, which was inefficient for miconazole nanogrinding, remained also inefficient to increase the drug solubility. Finally, the drug solubility was similar in all solutions containing SDS and the different cellulose ethers. Despite the important solubility of miconazole in SDS/HPC-LF, these excipients were selected for optimizing the nanogrinding of miconazole, as nanosuspensions are expected to be stable if the solubility of the drug substance is less than 1 mg/ml (Merisko-Liversidge et al., 2003).

Thus far, the data suggest that good indicators for the suitability of nanogrinding media for miconazole are (i) a low contact angle between the process solution and miconazole powder, and (ii) absence of microscopically visible agglomerates in the drug dispersions prior to nanogrinding, with the latter indicator being derived from subjective though consistent observations.

3.2. Optimization of miconazole nanogrinding

Miconazole nanogrinding was further optimized by a 2^3 DOE using SDS in combination with HPC-LF (Table 2). The three factors and their levels (concentration) were miconazole (5 and 20%,

Table 5
Miconazole particle undersize diameters d_{50} and d_{90} achieved by nanogrinding using the miconazole and the excipients HPC-LF and SDS according to a factorial 2^3 design with a repeated centre point. For comparison the particle size parameters before nanogrinding were: d_{50} = 27,000 nm; d_{90} = 49,000 nm.

Miconazole (%, w/w)	SDS (%, w/w)	HPC-LF (%, w/w)	Particle size d_{50} (nm) ± SD	Particle size d_{90} (nm) ± SD
5	0.05	1.25	164 ± 3	3300 ± 138
5	0.2	1.25	163 ± 0	3157 ± 62
5	0.05	5	150 ± 2	445 ± 21
5	0.2	5	169 ± 8	1044 ± 52
12.5	0.125	3.125	150 ± 1	536 ± 11
12.5	0.125	3.125	145 ± 1	507 ± 1
20	0.05	1.25	156 ± 1	1433 ± 28
20	0.2	1.25	153 ± 2	781 ± 57
20	0.05	5	140 ± 1	413 ± 10
20	0.2	5	152 ± 1	595 ± 20

w/w), SDS (0.05 and 0.2%, w/w), and HPC-LF (1.25 and 5%, w/w). High miconazole and HPC-LF concentrations promoted particle size reduction, as expressed by the d_{50} and d_{90} values (Table 5). Conversely, the effect of SDS concentration on d_{50} and d_{90} differed depending on the polymer concentration; increasing SDS concentration caused particle size (d_{90}) decrease at low HPC-LF concentration (1.25%, w/w), but particle size (d_{50} and d_{90}) increase at high HPC-LF concentration (5%, w/w). Both d_{50} and d_{90} values were fitted to a polynomial equation (Eq. (1)) after normalizing the coefficients, in order to evaluate the main effects and interactions affecting the d_{50} (Eq. (2); R^2 = 0.95) and d_{90} values (Eq. (3); R^2 = 0.99). The polynomial equations include only statistically significant variables, except the factor $-1.7W_{SDS}$ of Eq. (3), which could not be removed due to two significant interactions with SDS ($-115.7W_{MIC}W_{SDS}$ and $197W_{HPC\text{-}LF}W_{SDS}$). The obtained polynomial equations were:

$$d_{50} = 156 - 5.6W_{MIC} + 3.4W_{SDS} - 3.1W_{HPC\text{-}LF} + 4.4W_{HPC\text{-}LF}W_{SDS} \quad (2)$$

$$d_{90} = 1396 - 590.5W_{MIC} - 1.7W_{SDS} - 771.8W_{HPC\text{-}LF} - 115.7W_{MIC}W_{SDS} + 470.2W_{MIC}W_{HPC\text{-}LF} + 197W_{HPC\text{-}LF}W_{SDS} \quad (3)$$

where W corresponds to:

$$W = \frac{X_{i,j,k} - \text{centre point concentration}}{\text{concentration range}/2}$$

with $X_{i,j,k}$ being the corresponding concentrations (in %, w/w) of miconazole, SDS, and HPC-LF, respectively.

Miconazole and HPC-LF concentrations exerted the main effects on miconazole particle size reduction with the HPC-LF concentration being more important for reducing the larger particles (d_{90}). SDS concentration, on the other hand, exerted a detrimental effect on the d_{50} value ($p < 0.05$), but no significant effect on the larger particle size fraction (d_{90}) ($p > 0.05$). Besides the main effects, significant ($\alpha = 0.05$) positive interactions were observed between HPC-LF and SDS for both the d_{50} and d_{90} values as well as between miconazole and HPC-LF for the d_{90} value. By contrast, a significant negative interaction was determined between miconazole and SDS for d_{90} ($p < 0.05$).

The DOE was an important tool to explore and consolidate the effects of the formulation variables on miconazole particle size reduction. For confirming and further exploring the beneficial effects of high miconazole and HPC-LF concentrations and the interaction effect of HPC-LF and SDS, the milling experiments were extended using additional concentrations (Table 5 and Fig. 4). The additional miconazole concentrations of 12.5 and 25%, at fixed SDS (0.05%) and HPC-LF (5%) concentrations, yielded consistent particle size values in comparison with the previous experiments using 5 and 20% of drug substance (Fig. 4A and B). The variation of SDS concentration from 0 to 0.2%, at fixed concentrations of miconazole (12.5 or 20%) and HPC-LF (5%), revealed that a minimal amount of SDS (0.0125%) was necessary for efficient nanogrinding (Fig. 4C and D). However, increasing the SDS concentration from 0.0125 to 0.125% resulted in significantly ($\alpha = 0.05$) larger particle sizes (for d_{50} and d_{90}). As shown before in the DOE experiments, 0.2% SDS was detrimental for nanogrinding. This data is consistent with the fact that HPC-LF interacts with SDS above a critical aggregation concentration of 1.5 mM SDS (0.0433%), thereby probably reducing the adsorption of HPC-LF to the miconazole particles (Berglund et al., 2003).

For increasing the HPC-LF concentration above 5%, the miconazole concentration had to be kept at maximal 12.5% to avoid excessive suspension viscosity hampering the processing. Increasing the HPC-LF concentration from 1.25% to 6.25% (at 0.05% SDS) lowered the d_{50} and d_{90} to minimal values of approximately 150 nm and 400 nm, respectively (Fig. 4E and F). The data obtained with the higher HPC-LF and miconazole concentrations suggests that an adequately high viscosity may be one of the parameters that promote particle breakage. Conversely, an upper viscosity limit seemed to exist above which particle breakage was no further promoted.

To analyze the effect of viscosity on miconazole particle size reduction, the d_{50} and d_{90} values were plotted against the viscosity of all the starting suspensions used for nanogrinding (Fig. 5). The viscosity of the starting suspensions affected mainly the size reduction of the larger particles (d_{90}). The results indicate that a minimum viscosity of approximately 50–100 mPa s was required to obtain appropriate d_{90} values. On the other side, suspension viscosities exceeding 1300 mPa s hampered the processability of the suspensions in the actual stirred media mill.

3.3. Stability of miconazole nanosuspensions during storage

Storage of the nanosuspensions at 5 °C for 6 months generally caused only minor particle growth, in contrast to storage at 25 °C (Fig. 6). High miconazole concentration (20%; in presence of 0.05% SDS and 5% HPC-LF) stabilized much better the particle sizes during storage than the low (5%) drug substance concentration (Fig. 6A and B). Similarly, higher SDS concentration (0.2%) seemed to be preferable for storage of the nanosuspensions at 25 °C, as 0.05% SDS could not prevent substantial particle size growth, especially in the large particle size fraction (d_{90}) (Fig. 6D). Interestingly, microscopic observation of the nanosuspensions stored at 25 °C revealed that the increase of the large particle size fraction was probably mainly caused by crystal growth (Ostwald ripening). On the contrary nanosuspensions formulated without SDS presented an important fraction of particle aggregates, both before and after storage at 25 °C (data not shown). As for miconazole and SDS, the higher concentration of HPC-LF (5% versus 1.25%) attenuated the particle

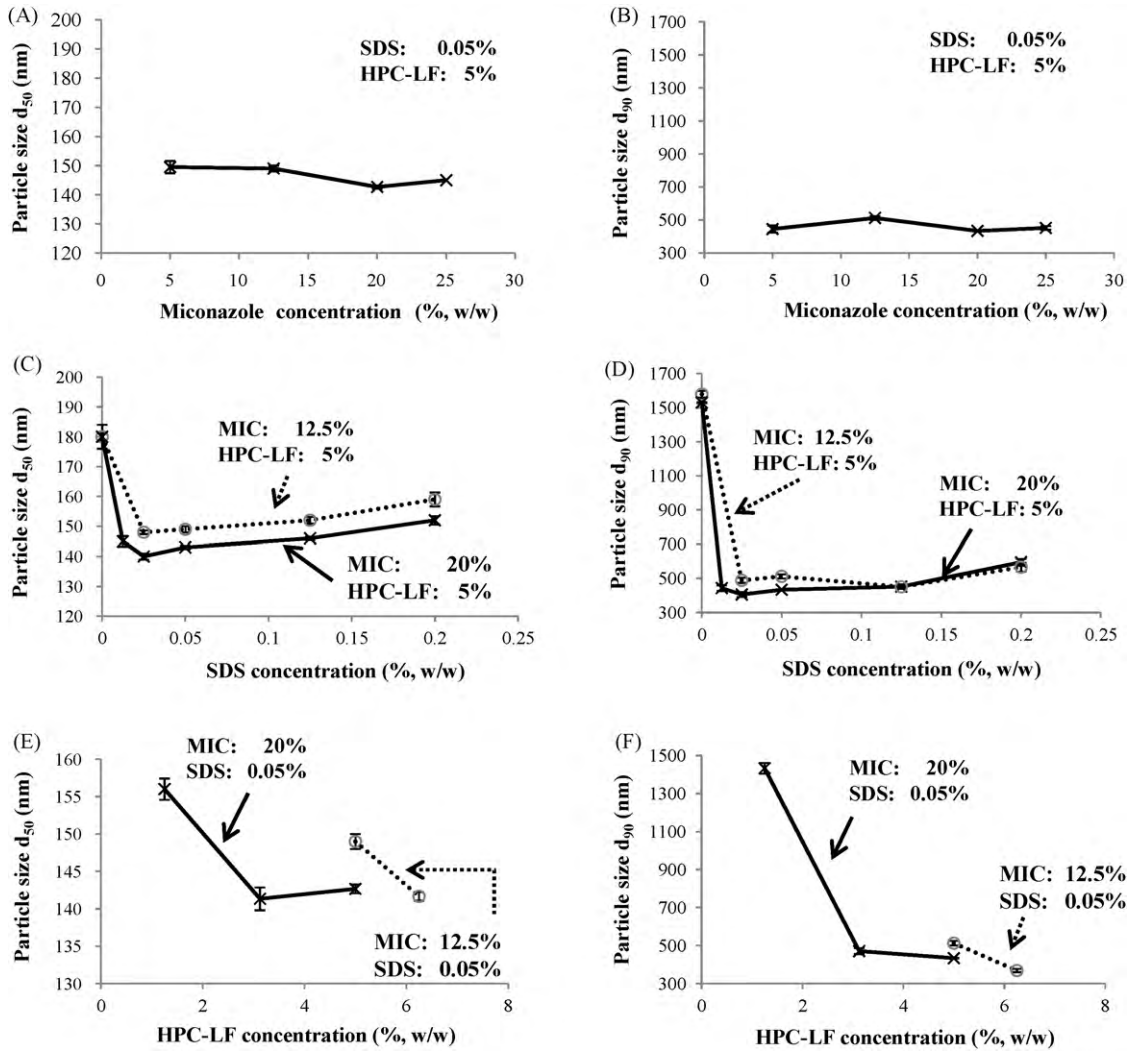

Fig. 4. Reduction of the miconazole particle sizes, expressed as d_{50} (panels A, C and E) and d_{90} (panels B, D and F), upon nanogrinding as a function of miconazole (A and B), SDS (C and D), and HPC-LF (E and F) concentrations.

size growth during storage at 5 °C and 25 °C (Fig. 6E and F). When comparing the differences in particle size growth of formulations stored at 5 °C and 25 °C, it appears that the steric stabilization, as provided by HPC-LF, was slightly more sensitive to the increased temperature than the electrostatic repulsion, as provided by the SDS (Rabinow, 2004).

Miconazole solubility and zeta-potential values were also examined before and after storage of the nanosuspensions at 5 °C and 25 °C, although data are available only for 3 months storage (Table 6). As expected, the higher miconazole (20%), SDS (0.2%), and HPC-LF (5%) concentrations all increased the miconazole solubility in the nanosuspensions before storage. The low drug solubility (1 μg/ml) in the medium containing low concentrations of SDS (0.05%) and HPC-LF (1.25%) must be ascribed partly to the limited solubilising capacity of both excipients at low concentration and partly to the fraction of large particles (d_{90} of approximately 1400 nm, see Table 5) found in this formulation in comparison to the other formulations shown in Table 6 (with d_{90} values in

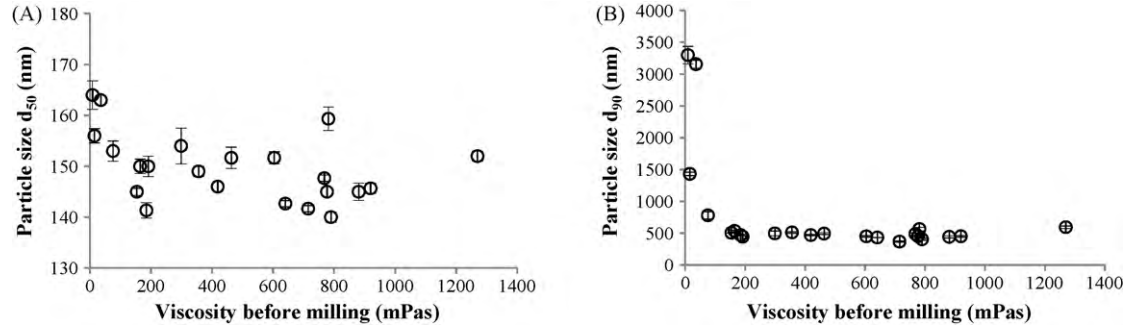

Fig. 5. Nanoground miconazole particle sizes, expressed as d_{50} (A) and d_{90} (B), as a function of the viscosity of the miconazole suspensions before milling.

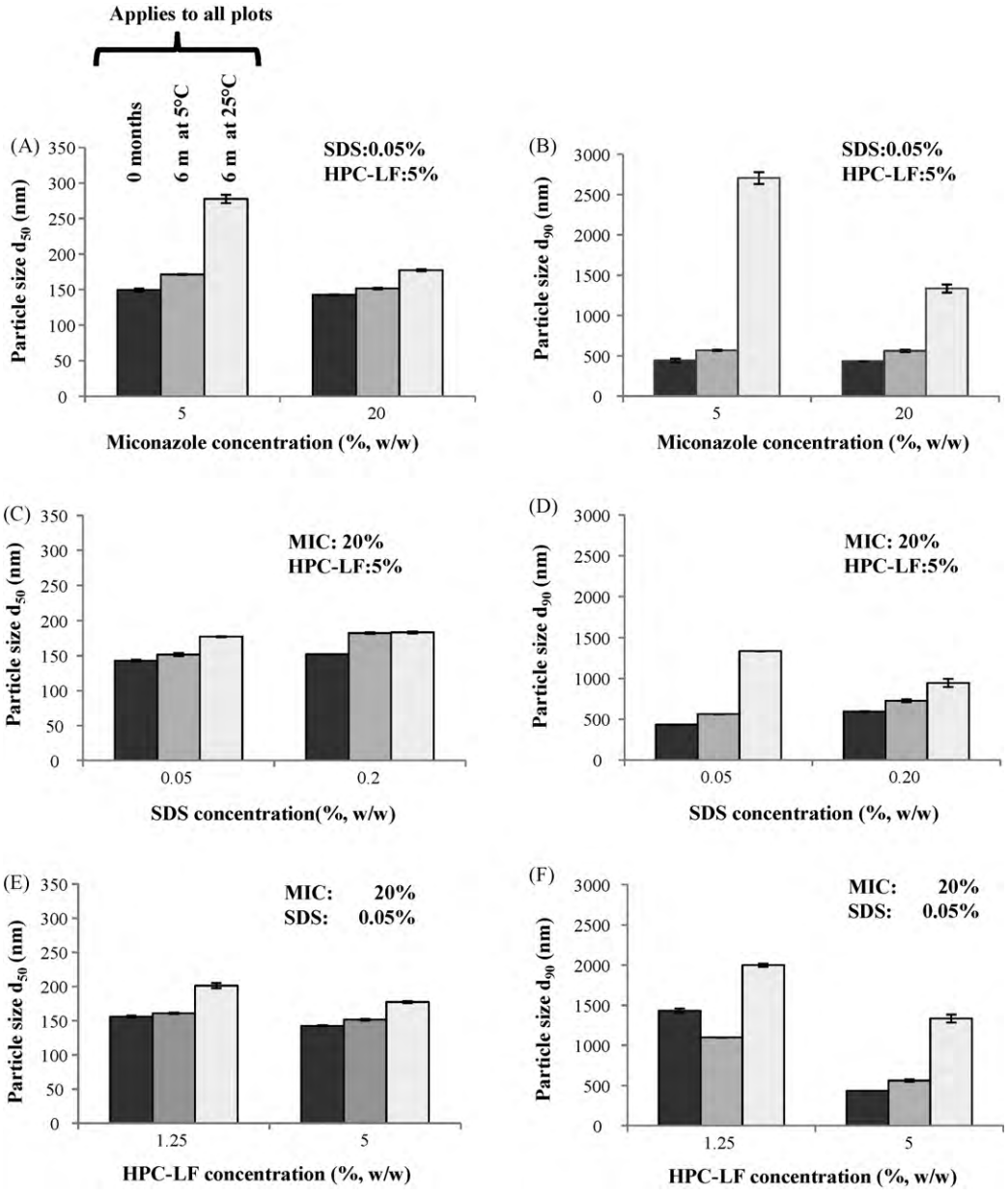

Fig. 6. Changes of the particle size parameters d_{50} (panels A, C and E) and d_{90} (panels B, D and F) of miconazole nanosuspensions during storage over 6 months at 5 and 25 °C. Effects of miconazole (A and B), SDS (C and D), and HPC-LF (E and F) concentrations.

the range of 400–600 nm, see Table 5). Upon storage, miconazole solubility tended to decrease slightly, although this could not be confirmed statistically. The zeta-potential values were mostly influenced by the SDS concentration of the nanosuspensions and did not change during the three month storage at 5 °C and 25 °C. Finally, neither the miconazole solubility nor the zeta-potential data could be related to the particle size stability results.

4. Discussion

Nanogrinding is a complex process requiring the selection of adequate formulation and process parameters to obtain appropriate particle size reduction and stability of nanosuspensions. In this study, we focused on the importance of formulation and related physical–chemical parameters. The selection of appropriate excipients is governed by two main functional criteria: (i)

Table 6
Miconazole solubility and zeta-potential in nanosuspensions before and after 3 months of storage.

Component (%, w/w)			Miconazole solubility (μg/ml) before and after 3 months of storage			Zeta-potential, $\bar{x} \pm$ SD, $n = 6$ (mV) before and after 3 months of storage[a]
Miconazole	SDS	HPC-LF	0 months	3 months at 5 °C	3 months at 25 °C	
5	0.05	5	21	38	21	−15 ± 0
20	0.05	5	58	47	15	−12 ± 1
20	0.20	5	86	52	56	−19 ± 1
20	0.05	1.25	1	1	1	−12 ± 1

[a] The zeta-potential did not alter during storage at 5 and 25 °C over 3 months.

wetting of the drug substance (Merisko-Liversidge et al., 2003), and (ii) steric and/or electrostatic stabilization of the nanoparticles (Rabinow, 2004). As the literature does not provide any rational criteria for the selection of excipients and process conditions, formulation development was done empirically. The initial screening of surface active and polymeric excipients revealed that HPC (both HPC-LF and HPC-EF) in combination with SDS was the most adequate, while poloxamer alone or the mixture of PVP and SDS were unsuitable for miconazole nanogrinding (Figs. 1 and 2). Good nanogrinding results were also obtained with the excipient mixtures of HPMC/SDS and HPC-LF/benzalkonium chloride (Fig. 3). While the possibility of substituting HPC by HPMC does not surprise, given their similar structure and properties, the suitability of both the cationic benzalkonium chloride and the anionic SDS suggests that the interaction between polymer and drug substance was more important than the interaction between surfactant and polymer. Such conclusion has already been made previously with SDS and benzethonium chloride (cationic surfactant), which were found to be equally effective for nanogrinding with both improving the interaction between polymer and 11 different drugs, thus promoting particle size reduction (Lee et al., 2008). However, the replacement of SDS by sodium docusate (also an anionic surfactant) in our study did not provide comparatively effective particle size reduction (Fig. 3). With sodium docusate, a relatively important fraction of coarse particles (diameters of 1–30 μm) remained in the nanosuspension after milling.

The results of the excipient screening study are in general agreement with reports in the literature, in particular regarding the usefulness of combining SDS and cellulose ethers (Rabinow, 2004; Lee et al., 2008; Van Eerdenbrugh et al., 2009). In a large formulation screening study using different drug substances, surfactants, and polymers, SDS promoted the nanogrinding in eight cases, but caused particle growth in five formulations (Lee et al., 2008). In fact, SDS was found efficient in combination with HPC, PVP and poloxamer 407 for the drug substances prednisolone acetate, nifedipin, hydrocortisone acetate, and itraconazole. SDS and HPC-LF are known to interact with each other and form polymer-surfactant aggregates (Evertsson and Nilsson, 1997; Berglund et al., 2003; Lee et al., 2008). Through such interaction, SDS may have facilitated the adsorption of HPC-LF on miconazole, thus promoting the formation of an entropic barrier preventing aggregation of nanoground drug particles (Choi et al., 2005). Moreover, the interaction between SDS and HPC-LF reduces the self-repulsion of the anionic SDS molecules thereby affording a greater particle surface coverage (Rabinow, 2004). The inferior suitability of PVP for producing nanosuspensions has already been described earlier, when HPC, HPMC, poloxamer, PEG, and PVP were compared as nanosuspension stabilizers at concentrations of approximately 17% relative to the concentration of drug substance (Lee et al., 2008). More recently however, higher PVP concentrations, i.e., 25–100% relative to the drug substance, produced more favourable results (Van Eerdenbrugh et al., 2009). The authors concluded that PVP is a valuable stabilizer when used at high concentration; use of high PVP concentrations is perfectly feasible thanks to the modest viscosifying capacity of PVP. With regards to poloxamer, previous studies described this polymer as highly versatile, as the hydrophobic PPO block adsorbs efficiently on hydrophobic surfaces of insoluble nanoparticles (Lee et al., 2008). Possibly other poloxamer types would be more suitable for miconazole nanogrinding than the poloxamer 188 used in the present study.

It is noteworthy that the screening experiments revealed two phenomenological parameters that predicted quite well the success of miconazole nanogrinding: (i) the contact angle between the drug substance and the stabilizer solution, which had to be relatively low; (ii) the dispersibility of the starting drug particles in the stabilizer solution, which had to be high and free of microscopically visible agglomerates.

Besides the selection of appropriate excipients, the optimization of their and the drug substance concentration in the suspension is equally important for nanogrinding (Table 5 and Fig. 4) and nanosuspension stability (Fig. 6). First, the concentration of drug substance in the suspension for nanogrinding needs to be sufficiently high to ascertain an elevated frequency of drug particle capture in the active grinding zone between beads; thereby, the milling energy of the beads is adequately transferred onto the drug particles (Stenger et al., 2005). Second, a minute amount of SDS (0.0125%) was required to provide adequate drug particle wetting for efficient nanogrinding. Higher amounts of SDS (>0.05%) were found to be detrimental for nanogrinding when HPC-LF was present at elevated concentration (Table 5 and Fig. 4). This observation might be explained by the competitive displacement of adsorbed HPC-LF by increasing SDS concentration (Evertsson and Nilsson, 1997; Berglund et al., 2003; Lee et al., 2008). In a model system using the hydrophobic poly(dimethyl siloxane) as adsorbant, HPC-LF adsorption was maximal at 1 mM SDS (0.03%, w/v) and decreased at higher SDS concentrations (up to 6 mM SDS; 0.17%) (Berglund et al., 2003). Displacement of the polymeric stabilizer from the drug surface likely lowers the steric stabilization provided by HPC-LF. This was highly detrimental in the case of miconazole as steric stabilization by HPC-LF was found to be crucial for efficient particle size reduction and nanosuspension stabilization, which is in agreement with other reports using other drugs (Lee, 2003; Ain-Ai and Gupta, 2008; Kobierski et al., 2009). The amount of polymeric excipient (e.g., HPC) not only determines its adsorption onto the drug substance particles, but also contributes to the viscosity of the suspension medium, thereby increasing the diffusion barrier for particle-particle interaction (Ploehn and Russel, 1990). For the miconazole nanogrinding, good results were obtained at HPC-LF concentrations of 3.125% and higher.

The viscosity of the miconazole suspensions for nanogrinding containing SDS and HPC-LF determined mainly the fraction of larger particles present after nanogrinding (Fig. 5). A minimal viscosity was required for efficient nanogrinding; under the present conditions, the lower critical viscosity was approximately 100 mPa s. Incidentally, the viscosity of the suspension resulted from the amounts of drug substance and polymeric excipient present in the suspensions. However, above an upper critical viscosity (not determined in this work), nanogrinding became less effective, which was explained by hindered movement of the milling beads (Kwade, 1999). Lower and upper critical viscosity values depend on the actual compound to be ground and the milling equipment and conditions. While in the present work, the upper critical viscosity appeared to be above 1000 mPa s (Fig. 5), such value was in the range of 170–470 mPa s for the inorganic fused corundum (α-Al_2O_3), used at concentrations above 20% (Stenger et al., 2005).

Finally, storage stability of the produced nanosuspensions in terms of particle size growth was acceptable when the nanosuspensions were stored at 5 °C for 6 months (Fig. 6). By contrast, when stored at 25 °C for 6 months, two types of larger particles became microscopically visible, i.e., particle agglomerates and larger individual crystals. Particle agglomerates were present in the absence of SDS, and crystal growth had occurred in the presence of SDS. The former phenomenon can be explained by the zeta-potential value that was close to zero in the absence of SDS, whereas crystal growth, also known as Ostwald ripening, was likely caused by increased drug substance solubility at 25 °C and increased SDS concentration (Verma et al., 2009). Under such supersaturation conditions, some of the dissolved

drug re-precipitated onto the larger particles with a lower surface energy.

5. Conclusions

As rational predictions for optimal excipients for nanogrinding are presently not available, this work provides simple empiric tools to orient experiments for fast achievement of stable nanosuspensions. Efficient particle size reduction by nanogrinding requires the use of excipients that provide proper wetting and physical stabilization (steric and electrostatic) of the practically water-insoluble drug substances. We found that a low contact angle between drug substance and dispersion medium in combination with an excellent agglomerate-free dispersibility of the micronized drug particles in the medium (before milling) can provide an indication of the suitability of excipients. For nanogrinding miconazole, the combination of 0.025–0.05% SDS and 5% HPC-LF was most suitable providing a synergistic effect for particle size reduction (d_{50} = 140 nm) and nanosuspension stabilization. SDS mediated wetting and facilitated the adsorption of HPC-LF onto the miconazole particles; HPC-LF adsorbed extensively onto the nanoparticles, thereby affording steric protection from agglomeration and crystal growth. The present findings may facilitate and accelerate the nanogrinding of other drug substances, as we have shown that prediction of particle size reduction and nanosuspension stability may be feasible, to some extent, from simple preformulation experiments. The study also emphasizes the importance of formulation development before process parameters should be optimized. Finally, appropriate particle size reduction and nanosuspension stability of practically water-insoluble drugs are important not only for enhancing the dissolution rate and bioavailability of the drug, but also for the safe use of the medicament by different administration routes such as the oral, nasal, ophthalmic and parenteral routes.

Acknowledgments

The authors thank Dr. Elke Walter for valuable comments and suggestions, and Sarah Barthold for experimental contributions.

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.

Augustijns, P., Van den Mooter, G., Van Eerdenbrugh, B., 2008. Top-down production of drug nanocrystals: nanosuspension stabilization, miniaturization and transformation into solid products. Int. J. Pharm. 364, 64–75.

Aulton, M.E., 2007. Aulton's Pharmaceutics. The Design and Manufacture of Medicines, 3rd ed. Churchill Livingstone, Edingburgh, pp. 65, 78.

Berglund, D.K., Przybycien, T.M., Tilton, R.D., 2003. Coadsorption of sodium dodecyl sulfate with hydrophobically modified nonionic cellulose polymers. 1. Role of polymer hydrophobic modification. Langmuir 19, 2705–2713.

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.

Crison, J.R., 2000. Biopharmaceutical aspects of water-insoluble drugs for oral drug delivery. In: Rong, L. (Ed.), Water Insoluble Drug Formulation. Interpharm/CRC, Boca Raton, pp. 98–108.

Evertsson, H., Nilsson, S., 1997. Microviscosity in clusters of ethyl hydroxyethyl cellulose and sodium dodecyl sulfate formed in dilute aqueous solutions as determined with fluorescence probe techniques. Macromolecules 30, 2377–2385.

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

Kesisoglou, F., Panmai, S., Wu, Y., 2007. 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.

Kobierski, S., Ofori-Kwakye, K., Müller, R.H., Keck, C.M., 2009. Resveratrol nanosuspensions for dermal application–production, characterization, and physical stability. Pharmazie 64, 741–747.

Kwade, A., 1999. Wet comminution in stirred media mills—research and its practical application. Powder Technol. 105, 14–20.

Lee, J., 2003. Drug nano and microparticles in solid dosage forms. J. Pharm. Sci. 92, 2057–2068.

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.

Liversidge, G.G., Cundy, K.C., Bishop, J., Czekai, D., 1992. Surface modified drug nanoparticles. US Patent 5,145,684.

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.

Muller, 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.

Ploehn, H.J., Russel, W.B., 1990. Interactions between colloidal particles and soluble polymers. Adv. Chem. Eng. 15, 137–228.

Rabinow, B.E., 2004. Nanosuspensions in drug delivery. Nat. Rev. Drug Discov. 3, 785–796.

Riley, S., 2006. Innovation in Drug Delivery. Business Insights.

Stenger, F., Mende, S., Schwedes, J., Peukert, W., 2005. Nanomilling in stirred media mills. Chem. Eng. Sci. 60, 4557–4565.

Sweetman, S., 2006. Martindale. The Complete Drug Reference, 35th ed. Pharmaceutical Press, London.

Van Eerdenbrugh, B., Vermant, J., Martens, J.A., Froyen, L., Van Humbeeck, J.V., Augustijns, P., Van den Mooter, G., 2009. A screening study of surface stabilization during the production of drug nanocrystals. J. Pharm. Sci. 98, 2091–2103.

Verma, S., Gokhale, R., Burgess, J.D., 2009. A comparative study of top-down and bottom-up approaches for the preparation of micro/nanosuspensions. Int. J. Pharm. 380, 216–222.