International Journal of Pharmaceutics 409 (2011) 260–268

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical Nanotechnology

Nanosuspension for improving the bioavailability of a poorly soluble drug and screening of stabilizing agents to inhibit crystal growth

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ARTICLE INFO

Article history:
Received 15 September 2010
Received in revised form 5 January 2011
Accepted 21 February 2011
Available online 1 March 2011

Keywords:
Nanomilling
Nanosuspension
Poorly soluble compound
Vitamin E TPGS
Rod shape crystals

ABSTRACT

The purpose of this study was to develop a nanosuspension of a poorly soluble drug by nanomilling process using wet media milling to achieve superior *in vitro* dissolution and high *in vivo* exposure in pharmacokinetic studies. A promising nanosuspension was developed with Vitamin E TPGS based formulation with particle size in the nano range. Although the formulation showed significant improvement during *in vitro* dissolution and *in vivo* plasma level, probably due to the strong hydrophobic interaction between Vitamin TPGS and the drug molecule, crystal growth was observed during stability studies. A systematic study was done with different combinations of solubilizer/stabilizer system in order to obtain a more stable nanosuspension. Hydroxypropyl methylcellulose (HPMC 3 cps) was found to stabilize the nanosuspension by better surface coverage due to stronger interaction with the drug as compared to other stabilizers used in this study.

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1. Introduction

Nanomilling process which reduces the particle size of active pharmaceutical ingredient (API) down to the sub-micron range is a popular technique in the pharmaceutical field for the delivery of poorly water soluble drugs. As the dissolution rate of the poorly soluble drug is proportional to the surface area, therefore nanomilling or nanosizing of poorly soluble drugs is a potential technique to achieve better *in vitro* dissolution and high *in vivo* exposure. Further, the saturation solubility of the drug also increases with reduction of particle size of the API. Finally the nanosystems have been known to reduce variability of drug absorption due to food effects for orally administered drugs (Kesisoglou et al., 2007).

Nanosuspensions are submicron colloidal dispersion systems which can be prepared by two basic methods. One is the bottom-up approach and other is the top down approach. Due to limitation of the bottom-up process during scale-up, the top-down techniques are frequently used as the potential technology for different commercial products. The top down process involves the particle size reduction of compounds using different wet milling techniques like media milling, microfluidization, high pressure homogenization, etc. The media milling comprises mechanical attrition of drug particles using milling media such as yttrium stabilized zirconium oxide beads of definite size range (Van Eerdenburgh et al., 2008).

During the milling process due to the change of Gibbs free energy, thermodynamically unstable nanosuspension is formed, which results in agglomeration or crystal growth due to Ostwald ripening. This in turn may impact dissolution and *in vivo* performance due to formation of larger particles with decreased surface area. Therefore proper selection of stabilizers is required during the preparation of nanosuspension to stabilize the nanoparticles by preventing them from aggregating due to the attractive force between the particles. In many cases a combination of stabilizers are more beneficial.

The most common approaches of stabilization are steric and/or electrostatic technique. Steric stabilization is achieved by adsorbing polymers onto the drug particle surface; whereas electrostatic stabilization is obtained by adsorbing charged molecules, both ionic surfactants or charged polymers, onto the particle surface (Van Eerdenbrugh et al., 2009). Common pharmaceutical excipients that are suitable for use as polymeric stabilizers include hydroxypropylcellulose (HPC), hydroxypropyl methylcellulose (HPMC 3 cps), polyvinyl pyrrolidone (PVP K30) and poloxamer (Pluronic F68 and Pluronic F127). Non-ionic surfactant stabilizers, such as polysorbate (Tween 80) and anionic surfactants such as docusate sodium (DOSS) or sodium lauryl sulphate (SLS) can also be used (Van Eerdenbrugh et al., 2009; Lee et al., 2008). The use of proper stabilizer in a naosuspension has to be done considering several factors. Polymer length and molecular weight of a polymer acts as the thermodynamic driving force for the physical adsorption on the surface of the particle. The higher the molecular weight of a polymeric stabilizer, the slower the rate of adsorption. Also high concentration of long chain polymers may lower the rate of dis-

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doi:10.1016/j.ijpharm.2011.02.051

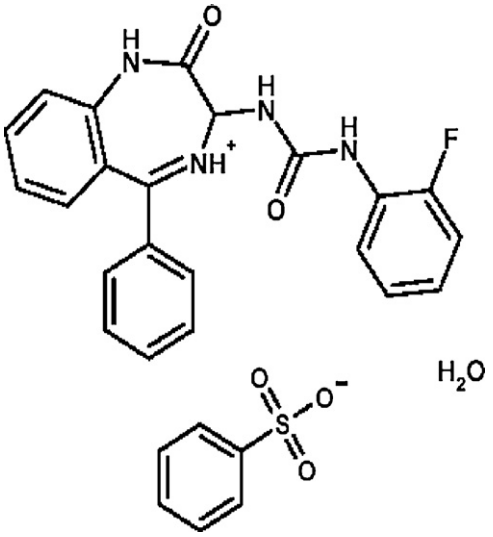

Fig. 1. Chemical structure of NVS-102.

solution which nullifies the benefit of nanomilling especially for poorly water soluble drugs. Further, stabilizers like sodium lauryl sulphate (SLS), Pluronic, at high concentration, sometimes offer challenge in producing patient friendly dosage form especially for paediatric group due to local gastric irritation.

In this study Vitamin E TPGS was used, which not only improved the *in vitro* dissolution and the bioavailability of the drug, but also helped to stabilize the nanosuspension during wet milling process by preventing the agglomeration of the drug substance. Studies were reported in the past about the importance of TPGS for improving the bioavailability of orally administered paclitaxel (Varma and Panchagnula, 2005) and nifedipine (Rajebahadur et al., 2006). The unique properties of Vitamin E TPGS as solubilizer, permeability enhancer and stabilizer led to the selection of this excipient for the nano system. Later during the stability study, an increase in particle size was observed due to crystal growth, justified the need of using an additional stabilizer. Further screening studies using different polymeric and ionic stabilizers were carried out to stabilize the nano system. HPMC 3 cps showed better capability to inhibit the crystal growth as compared to others.

The model drug used in this study was poorly water soluble with an equilibrium water solubility of 0.003 mg/ml. One of the important challenge during the nanomilling process was the morphology of the drug crystals, which was rod shaped having 15–20 μm mean particle size. Using proper process conditions, the drug crystals were milled down to nano particles.

2. Materials and methods

2.1. Materials

Compound NVS-102 from Novartis Pharma, has been used as a model drug in this study which is a rod shaped crystal of 15–20 μm mean particle size. The free base form of this drug is poorly water soluble with an equilibrium water solubility of 0.003 mg/ml. The melting point is 263 °C and its molecular weight is 388.4. The structure of the compound is shown in Fig. 1. The excipients used in this research, like Vitamin E TPGS was obtained from Eastman Co., UK, Pluronic F-68 and Pluronic F-127 were obtained from BASF and sodium lauryl sulphate (SLS) from Tensachem S.A., HPMC 3 cps was obtained from Dow chemical and PVP K-30 was obtained from BASF. Deionized water was used as dispersion media. Yttrium stabilized zirconium oxide beads with diameters of 0.1, 0.2 mm or 0.5 mm constituted the milling media. These were obtained from Netzsch. All other materials used were of analytical grade.

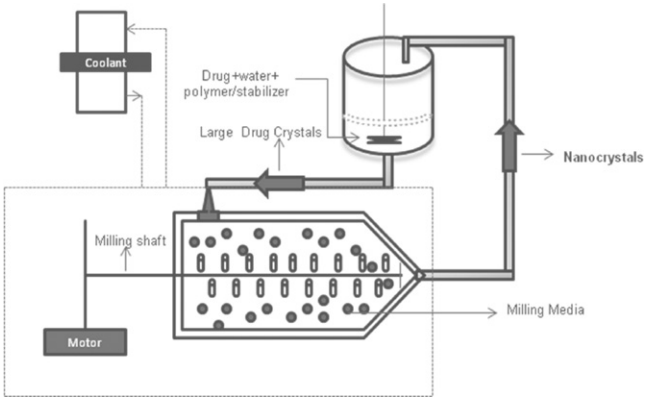

Fig. 2. The schematic diagram of nanomilling process.

2.2. Preparation of nanosuspensions

In this wet milling process, the drug and other ingredient were first dispersed in an aqueous-based solution, and the resulting suspension was wet milled with the grinding media (0.1 mm, 0.2 mm or 0.5 mm) using a Netzsch Labstar with Zeta agitator in recirculation mode. The processing temperature was maintained at less than 35 °C by controlled circulation of cooled water through the outer jacket. The pump speed was fixed at 250 rpm and the agitator speed was fixed at 2500 rpm. During the process the drug suspension was pumped into the milling chamber. High shear force generated during collision of the milling media with the solid drug and also due to the impact of drug crystals with the milling chamber provides the energy to fracture drug crystals into nano-sized particles (Fig. 2). The milled suspension passed through the screen (which retained the grinding beads) and went back to the suspension beaker and then again re-circulated to the nanomill. The process continued until desired particle size was reached. Sampling was done at regular intervals for particle size analysis.

2.3. Characterization of nanosuspensions

2.3.1. Particle size determination

Processed suspensions were characterized by intensity-weighted particle size using Beckman Coulter particle size analyzer (Model N4). A drop of the nanosuspension sample (about 5 μl) was diluted with 5 ml of DI water. The cuvette was shaken for about 10 s by hand and placed immediately inside the sample holder of particle size analyzer. Once the required intensity was reached, analysis was performed to get the mean particle size and polydispersity index (PI).

2.3.2. Scanning electron microscope

The samples were evaluated by using JEOL JSM6301 FXV scanning electron microscope (JEOL, Peabody, MA, USA). One drop of nanosuspension without any further dilution was air dried on the aluminium stubs and then coated with Palladium before imaging. The images were taken on Joel, SEM and then the images were transferred to Clemex's Vision professional Edition version 6.0.004C. The scale bar was calibrated accurately. Then in each case 4-5 images from different locations (bitplanes) were used for particle size distribution. Number of particles in each images varied from 250 to 400 particles. The sizes were reported in terms of length.

Table 1
Composition of nanosuspension.

Formulation code	Formulation composition (% w/v)							
	Batch size (ml)	Drug	Vit. ETPGS	Pluronic F68	Pluronic F127	SLS	HPMC 3 cps	PVP K-30
NS1	600	5.00	5.00	–	–	–	–	–
NS2	600	5.00	3.00	2.00	–	–	–	–
NS3	600	5.00	3.00	–	2.00	–	–	–
NS4	600	5.00	5.00	–	–	1.00	–	–
NS5	600	5.00	5.00	–	–	–	1.00	–
NS6	600	5.00	5.00	–	–	–	–	1.00

2.3.3. X-ray powder diffraction

X-ray powder diffraction analysis was performed on Bruker D8 Advance, controlled by Diffrac plus XRD commander software. The sample was prepared by spreading powder samples on a PMMA specimen holder rings from Bruker and were scanned from 2 to 40° 2θ at the rate of 2°/min with 0.02° step size and 0.6 s/step at 40KV and 40 mA. The divergence and anti-scattering slits were set to 1° and the stage rotated at 30 rpm. Data analysis was performed using "EVA Part 11" version 14.0.0.0.

2.3.4. In vitro dissolution study

The prefilled capsules with nanosuspension were analyzed by 2 step dissolution process using pH 2 media followed by pH 6.8 media, using Apparatus 1 (basket) which were rotating at 100 RPM, The sample was filtered through 0.2 μm PTFE filter before being analyzed. The 1st 1 h of the dissolution study was conducted in pH 2 media and later 1 h was conducted in pH 6.8 media.

2.3.5. Pharmacokinetic study

The drug was formulated and prepared as intralipid suspension of coarse particles and nanosuspension. The two formulations were dosed to the fasted male beagle dogs (n = 3/group) at a dose of 20 mg/kg by oral administration. Plasma samples were analyzed for parent compound by LC–MS/MS by DMPK/BA, East Hanover, NJ. The lower limit of quantification was 10.0 ng/ml. Pharmacokinetic analysis was performed using WinNonlin© Professional (version 5.2) by DMPK-nonclinical PK/PD.

3. Results and discussions

3.1. Effect of nanomilling on particle size of drug compounds in presence of different stabilizers

Different polymeric stabilizers and solubilizer were evaluated during this study were shown in Table 1. For most of the variants, a significant reduction in particle size was observed within first few hours of wet milling as shown in Fig. 3. For screening of the different formulations, 3–4 h nanomilling was conducted and the particle size were compared.

The most effective particle size reduction was observed with the formulation containing 5%Vitamin ETPGS alone and also with 5% Vitamin ETPGS and 1% HPMC 3 cps. In another set of studies, a part of Vitamin ETPGS was replaced by Pluronic which led to the formulation having 3% Vitamin ETPGS and 2% Pluronic. While comparing the different grades, Pluronic F-68 produced smaller particles compared to Pluronic F-127 after milling process. Finally significantly larger particles were observed when 1% SLS was added to the Vitamin E TPGS formulation, probably due to agglomeration during the process. The details of these observations are explained in the later sections.

One of the most important characterization studies of nanosuspension was the particle size of the drug crystals. The particle size of the nanosuspension was determined using various analytical techniques like fixed-angle routine photon correlation spectrometer, PCS (N4 plus equipment) and also by scanning electronic microscopy (SEM). The mean values were collected from PCS analysis, which generally yields accurate results in the nano range and also fast and easy to perform. However this technique always cannot able to analyse larger particles in micron range, therefore SEM was used for detecting larger particles in the nanosuspension. Infact comparatively larger mean particle size was observed when SEM method was used. This could be explained due to the broad distribution of the particle sizes present in the system which can be visualized in the SEM pictures. Therefore, the particle size analysis was done by both methods (PCS and SEM) during the screening study. Also in order to study the effect of any possible dissolution on drug compound during particle size analysis by PCS method, study was done by diluting the nanosuspension with unsaturated, partially saturated and completely saturated disperison media. The results showed no significant effect of dilution on the particle size analysis. Although the polydispersity of particle size measured by

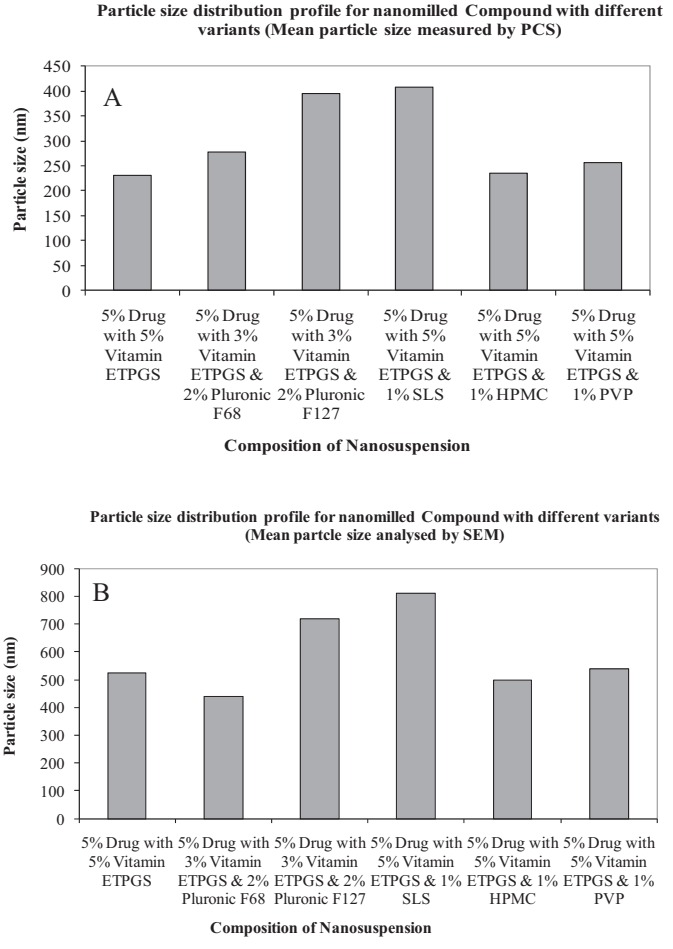

Fig. 3. Particle size of drug substance after 3 h of nanomilling for different formulations ((A) mean particle size measured using PCS; (B) mean particle size measured using SEM).

Table 2
Effect of bead size on particle size reduction for nanomilled compound.

Milling time (h)	Size of beads		
	Nanomilling with 0.1 mm beads	Nanomilling with 0.2 mm beads	Nanomilling with 0.5 mm beads
1	384.0 (0.484)	286.4 (0.231)	497.2 (0.284)
2	277.8 (0.347)	248.5 (0.123)	409.3 (0.026)
3	263.4 (0.210)	230.2 (0.257)	375.3 (0.335)

PCS were relative high (less than 0.5) during nanomilling due to the presence of larger particles, however after the completion of process for most of the formulations polydispersity index (PI) were found to be less than 0.2. Also during stability studies, PI was less than 0.2 for all the formulations.

3.2. Effect of bead size on nanomilling

The efficiency of the milling depends on the intensity of grinding energy and the size of the milling media is an important factor to control the efficiency of the process. In order to improve the efficiency of the milling process, a study was conducted to optimize the size of the milling media. After nanomilling for about 3 h using different size beads (Table 2), 0.1 mm and 0.2 mm beads produced most efficient particle size reduction compared to 0.5 mm beads. 0.2 mm bead size was selected for all nanomilling studies. Also another interesting result observed during the milling process was the significantly fast reduction of the mean particle size during the 1st one hour (from 15 to 20 μm to less than 500 nm). Subsequently the rate of the particle size reduction was slowed down. This may be probably due to the fact that, mostly deagglomeration of drug particles took place initially, followed by the breakage of the crystals due to cleavage and fracture. The later process usually requires more mechanical stress.

3.3. Nanosuspension with Vitamin ETPGS

3.3.1. Effect of Vitamin ETPGS during nanomilling process

Vitamin E-TPGS (TPGS), which is non-ionic water soluble derivative of Vitamin E found to enhance the solubility and hence the bioavailability of many poorly soluble drug compounds. Previous studies had shown that TPGS improved the bioavailability of orally administered Paclitaxel which have low solubility or limited permeability. (Varma and Panchagnula, 2005). Also in another study, solubility was improved for Nifedipine when TPGS was used in the formulation. (Rajebahadur et al., 2006).

The purpose of using Vitamin E TPGS was to improve the bioavailability of the drug and also to produce stabilization effect to the drug crystal by absorbing on the drug surface. The amount of Vitamin E TPGS used for the nanosuspension was 5% v/v, which is about 100% of drug concentration in the suspension. Previous studies had demonstrated Vitamin E TPGS to be effective in stabilizing the nanosuspension at high concentration of 25–100% due to its low viscosity and high surface activity properties (Van Eerdenbrugh et al., 2009). From the present research work, it was observed that Vitamin E TPGS produced relatively smaller particle size as compared to Pluronic (both F68 and F127) or sodium lauryl sulphate (SLS). Finally since this formulation was developed for paediatric dosage form, Vitamin E TPGS was considered to be a better option compared to Pluronic or SLS because of less gastric irritation property.

One of the other benefits of using TPGS in Nanosuspension is its low viscosity as compared to other stabilizers like HPMC, PVP, HPC and even Pluronic. After nanomilling for few hours, the particle size reduced gradually to around 200 nm. Nanosuspension prepared with Vitamin E TPGS is of relatively low viscosity which significantly reduces the milling time due to high attrition rate of the beads. The low viscosity of TPGS significantly reduces the milling time due to high attrition rate of the beads as a result of less resistance to the movement of the beads. Prolonged milling

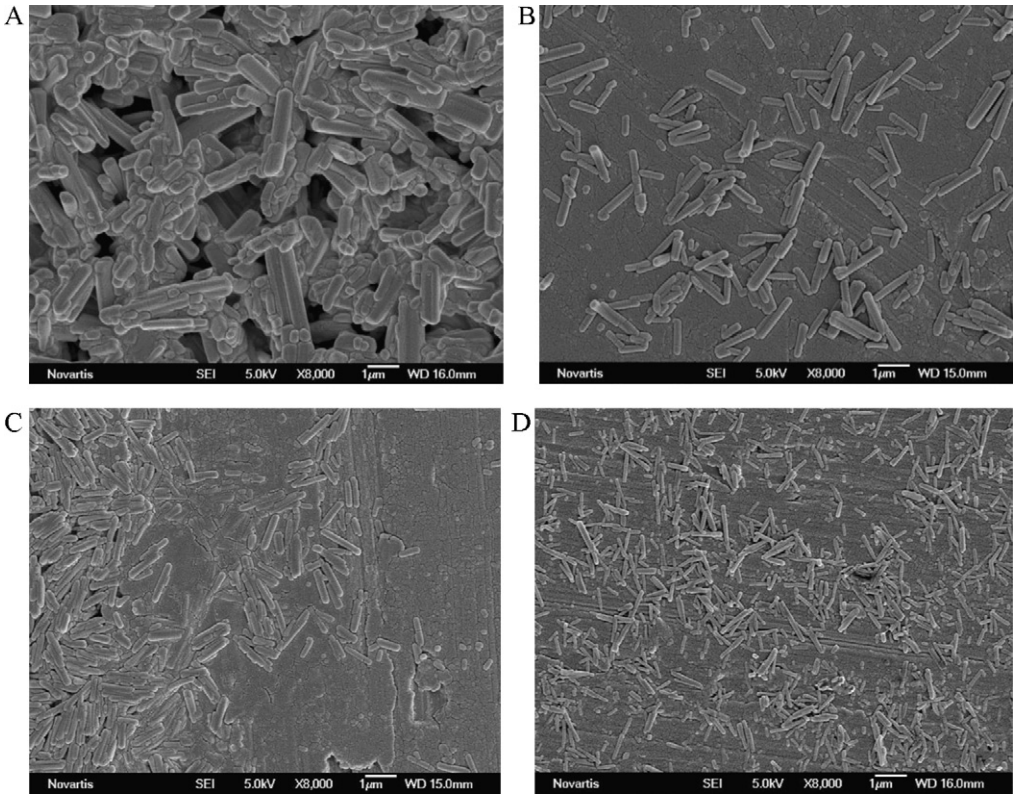

Fig. 4. Scanning electron images of the rod shaped drug crystal after different grinding time. ((A) start, (B) after 30 min grinding, (C) after 1 h grinding, (D) after 3 h grinding)

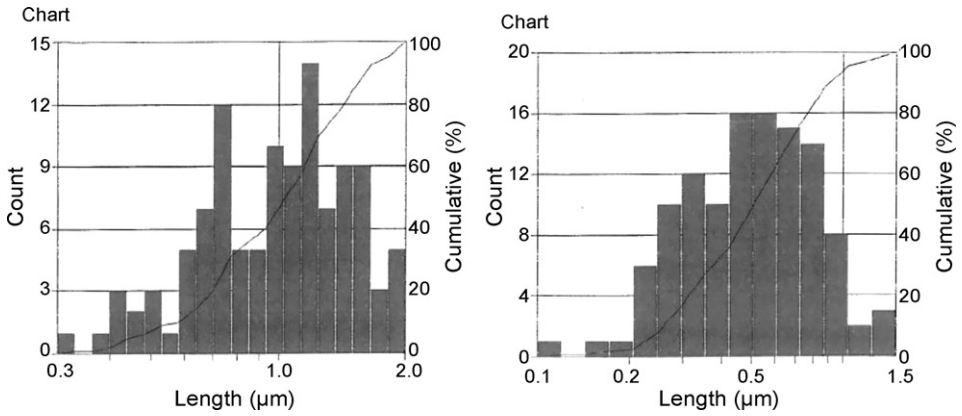

Fig. 5. Particle size distribution of drug crystals obtained from scanning electron images after different grinding time ((A) after 30 min grinding, (B) after 3 h grinding).

time is sometimes responsible for increased thermal energy which is sometime responsible for degradation of the thermosensitive drugs.

During the *in vitro* release, 5% TPGS nanosuspension gave promising dissolution profile, discussed in the later section. Thus Vitamin E TPGS had been shown to be a unique stabilizer which not only brings down the particle size of the drug to nano range but also improves the *in vitro* dissolution, due to strong hydrophobic interaction between Vitamin E TPGS and drug molecule. The amount of Vitamin E TPGS studied during the initial research, varied from 1.25% to 5.0% (w/v) which is equivalent to 25–100% (w/w) of drug concentration. No studies were conducted above 5% because of possible challenge during down streaming or drying process as described in the earlier section. While studying the effect of its concentration on the drug particle size after processing, it was noticed that the mean particle size of drug after 3 h nanomilling with 5% TPGS was smaller (523 nm) as compared to 2.5% TPGS (901 nm) and 1.25% TPGS (1200 nm) as analyzed by SEM. Obviously it indicated that 5% TPGS has more stabilization effect on drug and based on this observation 5% TPGS was selected for further studies. In another separate study, when HPMC 3 cps was used as stabilizer, 2.5% TPGS also produced much smaller particle size, which will be discussed in the 2nd part of this manuscript.

The morphology of the drug compound used in this study was rod shaped crystal. While studying the grinding mechanism it was observed that the crystals shifted to significantly smaller size within 30 min of grinding, after which there was slow down in the rate of size reduction (Fig. 4). The possible reason is described in the previous section. Also the intermediate size crystals formed during the grinding process consisted of broad range of particles which mainly occured due to the cleavage and fracture mechanism. Since the amount of fines were less, abrasion mechanism was less likely to occur. Although no significant reduction of particle size was observed after 3 h, however when the particle size distribution study was performed from the image obtained from SEM, the range of particle size was narrowed down after 3 h compared to 30 min (Fig. 5) due to the fracture of bigger crystals.

3.3.2. Crystal properties of nanosuspension

One of the drawbacks of nanomilling is the probability of drug substance to convert to amorphous state. The high speed of the mill and also the heat generated during the process is responsible for the form change. Since the mobility of the drug is higher in amorphous phase as compared to crystalline phase, therefore crystalline nanoparticles are preferable to avoid stability issues. The XRPD study was performed with freeze dried nanosuspension and the results showed no change of crystallinity of the drug substance (Fig. 6).

3.3.3. In vitro dissolution study for nanosuspension

In vitro dissolution studies were performed to rank-order the suitable variant for *in vivo* PK study. A comparative dissolution study was performed with nanosuspensions having different particle size of the drug compound. The study was performed with

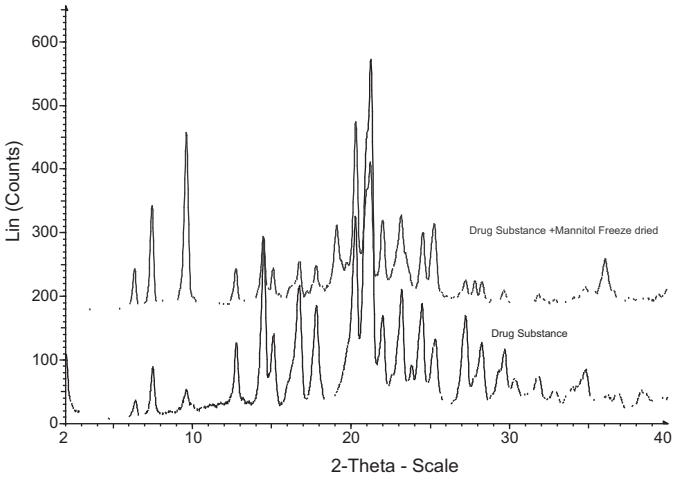

Fig. 6. XRPD of freeze dried nanomilled compound showing crystalline property of drug is retained.

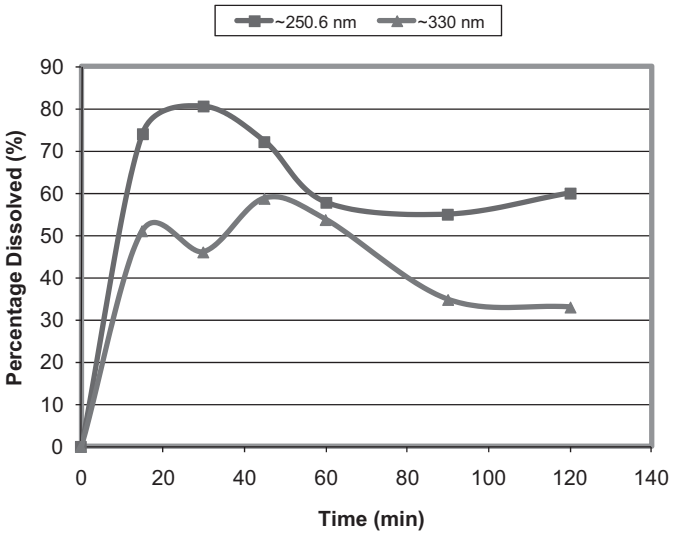

Fig. 7. Effect of particle size of drug in nanosuspension on dissolution.

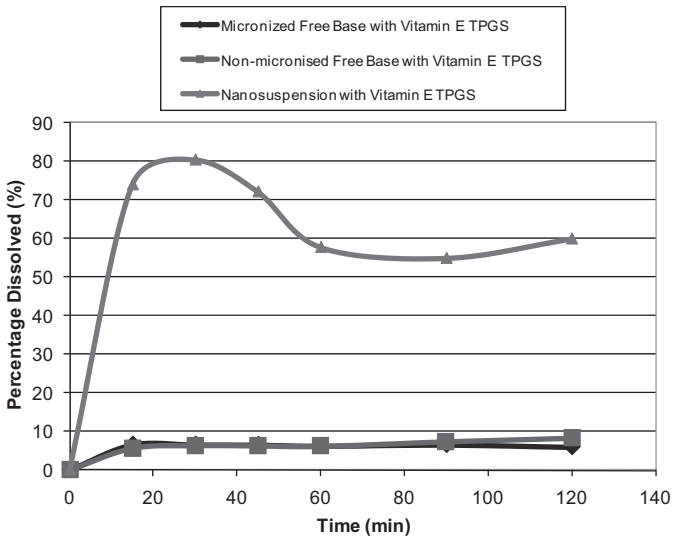

Fig. 8. Comparison of dissolution profile of milled drug (nano range) vs. coarse drug (micronized and non-micronized) each containing Vitamin E TPGS.

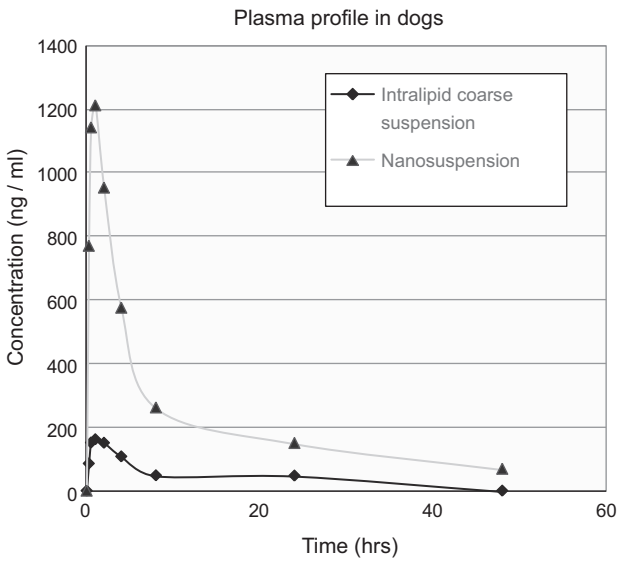

Fig. 9. Pharmacokinetic profile of drug compound in dog using nanosuspension versus intralipid coarse suspension.

same formulation prepared with 5% Vitamin E TPGS under similar processing conditions. The nanosuspension samples used for this study had the mean particle size of 250.6 (PI-0.133) nm and 330 nm (PI-0.407). Initial rate of dissolution was directly proportional to the nanosuspension particle size (Fig. 7) which confirmed the discriminatory power of dissolution method. The drop in dissolution can be explained by the switch of dissolution media from pH 2 to pH 6.8 which was performed at 60 min. Since the compound is a free base, higher solubility would be expected in the acidic pH (pH 2) and some amount of precipitation resulting in a lower dissolution value would be expected when the pH is increased to 6.8.

When dissolution study was compared with nanosuspension containing Vitamin E TPGS and non-micronized and micronized drug formulations also with Vitamin E TPGS, significantly faster drug release were observed for the nanosuspension formulation (Fig. 8).

3.3.4. *In vivo* study for nanosuspension

The *in vivo* study conducted in dog has shown superior exposure compared to non-micronized coarse suspension (Fig. 9). The AUC of nanosuspension was increased by about 9 fold compared

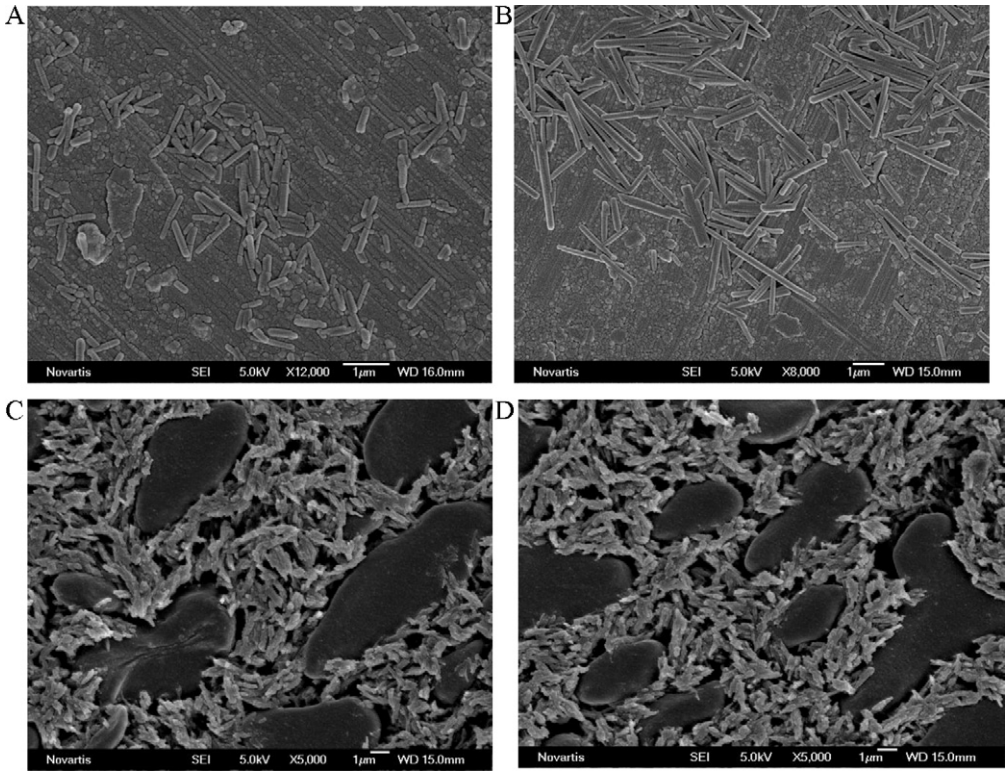

Fig. 10. SEM of nanomilled compound to show crystal growth ((A) SEM picture of 5% Vitamin E TPGS nanosuspension at initial time, (B) SEM picture of 5% Vitamin E TPGS nanosuspension after 3 m, (C) SEM picture of 5% Vitamin E TPGS + 1% HPMC 3 cps nanosuspension at initial time, (D) SEM picture of 5% Vitamin E TPGS + 1% HPMC 3 cps nanosuspension after 3 m).

Table 3
Crystal growth of drug during short time stability (nanosuspension with 5% TPGS).

Time	Mean particle size (nm) (PCS)
Start	230.2 (0.257)
1 month	312.0 (0.163)
3 months	477.8 (0.155)

to the coarse suspension. Also the C-max of nanosuspension was increased by about 5 fold.

3.4. Effect of other stabilizers on the particle size of drug during nanomilling process and also during short term stability

3.4.1. Effect of HPMC 3 cps

A short term stability study was performed for evaluating the comparative stabilization efficiency of different stabilizers used in the nanosuspension system. The stability study was performed at 2–8 °C. and the particle size of the samples was tested at initial, 1 month and 3 months time points.

During the stability study of the formulation having only 5% TPGS, increase of particle size was observed (Table 3). SEM results indicated that the increase of particle size of drug compound was most probably due to increase of the crystal growth instead of agglomeration (Fig. 10A and B). It was reported that the instability of nanosuspension may be caused due to nucleation and particle growth. This observation justified the need of additional stabilizer to overcome the problem by providing steric barrier since Vitamin E TPGS alone may not able to stabilize the drug completely. When

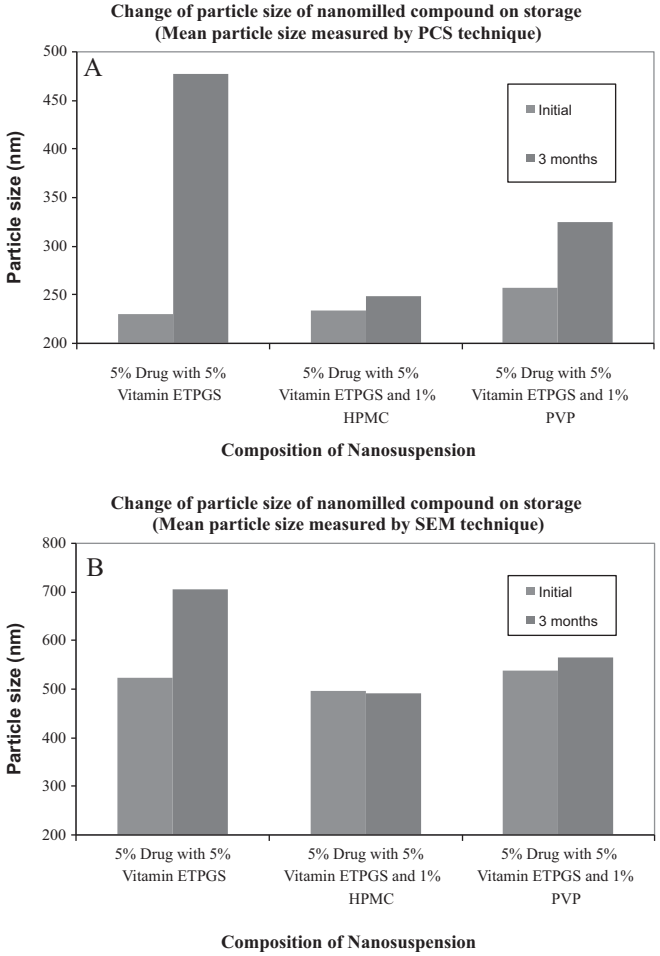

Fig. 11. Effect of crystal inhibitors to arrest the growth of particle size of drug during stability.

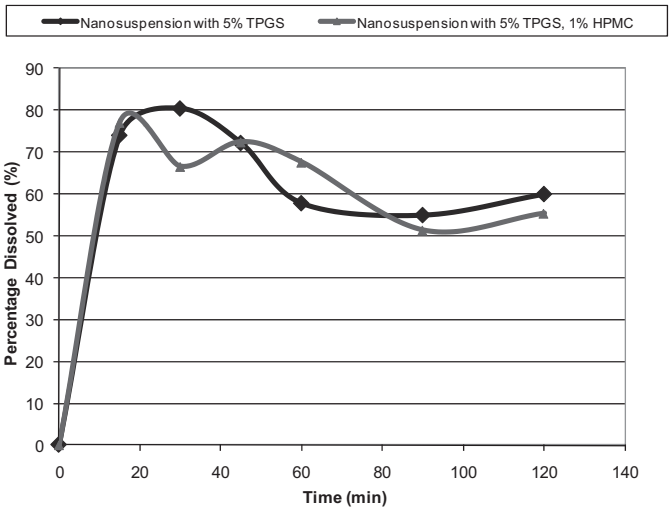

Fig. 12. Comparison of HPMC 3 cps on dissolution profile of nanomilled drug compound using Vitamin E TPGS study ((A) mean particle size measured using PCS; (B) mean particle size measured using SEM).

1% HPMC 3 cps (hydroxypropylcellulose) was used in suspension as polymeric stabilizer (20% w/w of drug content), no significant increase of particle size occurred probably due to its inhibition effect on crystal growth (Fig. 11). HPMC 3 cps polymer probably got adsorbed onto drug crystals due to interaction of the hydrophobic (methoxyl) and hydrophilic (hydroxypropyl) groups with the drug and provided steric stabilization (Frantzen Christer et al., 2003).

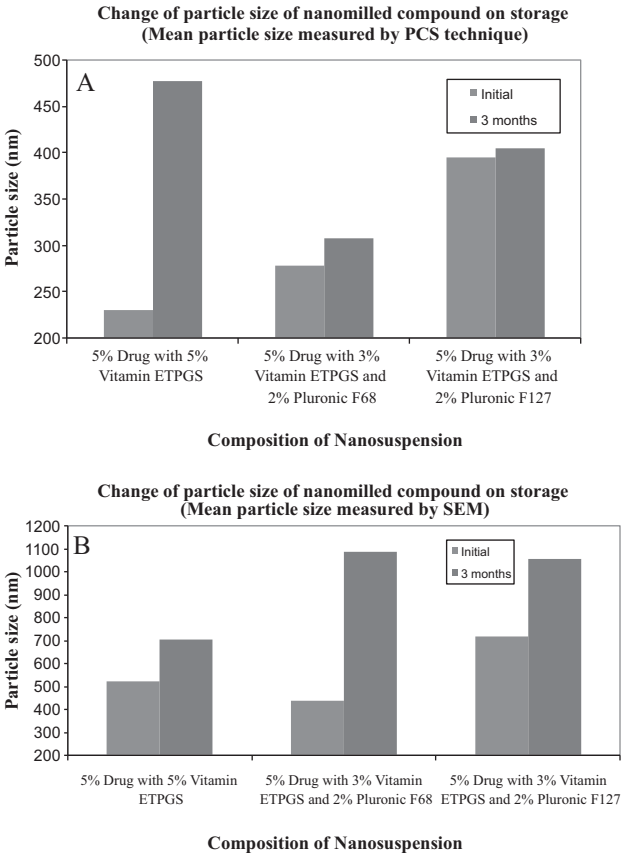

Fig. 13. Effect of various grades of Pluronic to inhibit the growth of particle size of drug during stability study ((A) mean particle size using PCS; (B) mean particle size using SEM).

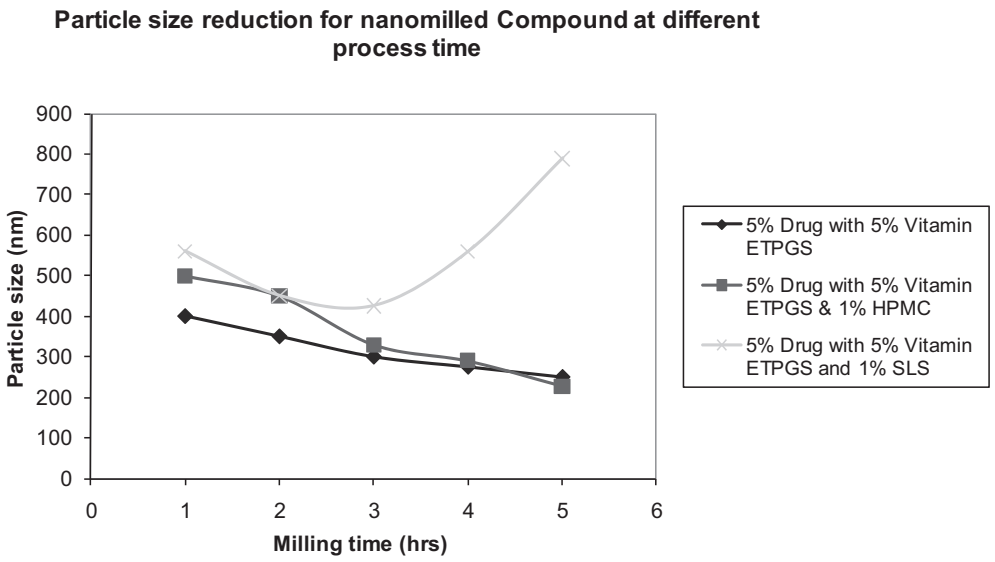

Fig. 14. Effect of different formulations on particle size reduction for nanosuspension during milling.

The absorption of HPMC 3 cps polymer on the surface of the nuclei of the drug resulted in crystal growth inhibition as observed during the SEM study (Fig. 10C and D). When HPMC 3 cps was replaced by PVP K-30, crystal growth was inhibited to some extent but not completely (Fig. 11). Therefore it can be concluded that HPMC 3 cps interacted more strongly with the drug compared to PVP K-30 and gave a better surface coverage. The high affinity of HPMC 3 cps on the drug molecule can be explained due to its open chain like structure whereas PVP K-30 has more compact or coil shaped structure. This hypothesis was well explained by Verma et al. (2009) while comparing the above 2 polymers using AFM study.

Finally while conducting *in vitro* dissolution, no significant change in drug release was observed for Nanosuspension prepared with 5% Vitamin E TPGS and 1% HPMC 3 cps as compared to Vitamin E TPGS alone (Fig. 12).

3.4.2. Effect from various grades of Pluronic

Pluronic is a block co-polymer, responsible for the hydrophobic interaction with the drug molecule. This was also evaluated as a steric stabilizer. A mixture of stabilizer is sometimes required to obtain a stable nanosuspension (Dolenc et al., 2009). Also the mixture of TPGS and Pluronic was shown to be a potential particle size growth inhibitor in the past studies (Dai et al., 2008). When a part of Vitamin E TPGS was substituted by Pluronic F-68 or Pluronic F-127, the crystal growth was inhibited to some extent. In this study, a part of Vitamin E TPGS was replaced with Pluronic F-68 or Pluronic F-127 to gave a final composition of 3% TPGS and 2% Pluronic. Both nanosuspension variants formulated with Pluronic F-68 and Pluronic F-127, achieved nano-size particles. However the particle size achieved with Pluronic F-68 were smaller as compared to Pluronic F-127 initially, at $t = 0$ time point (Fig. 13). As the molecular weight of Pluronic F-68 used in this study was more than the F-127, the rate of absorption of F-68 on the drug surface was faster as compared to F-127 (Choi et al., 2008).

However, during storage, the rate of crystal growth of the drug was slightly faster in case of Pluronic F-68 as compared to Pluronic F-127. This may be due to the fact that the crystal growth inhibition is mainly due to the hydrophobic polypropylene oxide group (PPO) in the Pluronic polymer. As the influence of the strength of hydrophobic hydrogen bonding on the crystal growth is a kinetic process, it gets manifested over time during the stability study. Similar observation was also reported by (Dai et al., 2008).

3.4.3. Effect of SLS

Besides evaluating the steric stabilization of the nanosuspension, electrostatic stabilization was studies using sodium lauryl sulphate (SLS). 1% of this surfactant was mixed with 5% TPGS. Although, there was initial reduction of particle size during the first few hours of milling, the particle size increased thereafter (Fig. 14). To investigate this unique effect of SLS, solubility study was performed. It was observed that the drug solubility was sig-

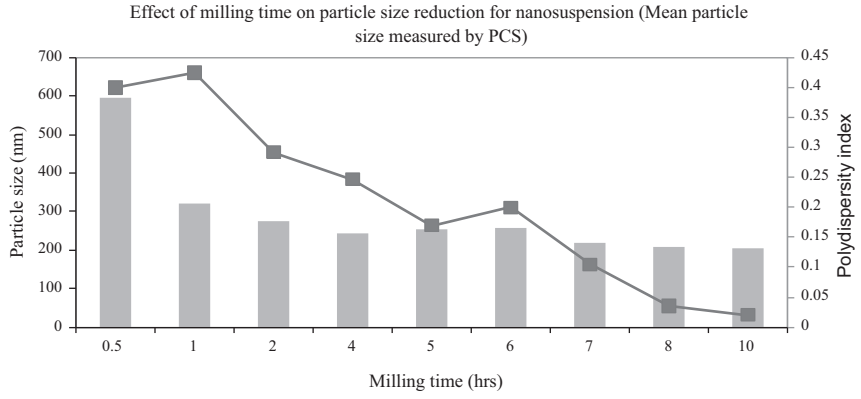

Fig. 15. Effect of milling time on particle size reduction for nanosuspension.

nificantly high in presence of SLS as compared to TPGS (10% SLS – 2.517 mg per ml and 10% TPGS – 0.51 mg per ml).

As SLS significantly increased the intrinsic solubility of the drug, there is a high chance that during the processing, Ostwald ripening can occur. This type of observation can be explained by Liftshitz, Slyozov and Wagner (LSW) theory (Lindfors et al., 2006).

As per the LSW theory, rate of Ostwald ripening can be explained by the following equation: $D(d)^3/dt = 64\lambda Vm^2 cD/9RT$, where λ is the interfacial tension, Vm is the molar volume of the dispersed compound, c is the bulk solubility, D is the diffusion coefficient in the solvent, R is the gas constant and T is the absolute temperature. As per this theory, most of the parameters are constant except the interfacial tension and bulk solubility. Since the bulk solubility of the drug increased by addition of 1% SLS, the rate of Ostwald ripening also increased. Although SLS was responsible for the drop in interfacial tension during the initial stage, however the increase of surface area with increase of milling time increased the interfacial tension which leads to time dependent Ostwald ripening during the nanomilling process.

3.5. Particle size reduction of nanosuspension as function of milling time

During the above studies, 3 h milling was performed for fast comparative screening of stabilizers. After selecting the most optimized variant with 5% Vitamin E TPGS and 1% HPMC, nanomilling was performed for extended time (10 h). The result showed not much significant decrease of particle size with extended milling time (Fig. 15). However decrease of polydispersity index (PI) was observed with time, which confirmed that with prolonged milling time, remaining larger particles in the nanosuspension were broken down into smaller particles.

4. Conclusion

A promising nanosuspension formulation was developed with Vitamin E TPGS, which produced better results compared to non-micronized formulation during *in vitro* dissolution study and *in vivo* dog PK study. The optimization of the formulation with HPMC 3 cps resulted in inhibiting crystal growth during stability as compared to other stabilizers like PVP K-30, Pluronic or SLS. Also Vitamin E TPGS is considered to be a better option compared to Pluronic or SLS because of the potential gastric irritation property of Pluronic or SLS at higher concentration especially in pediatrics dosage form. Further studies are in progress with lower concentrations of Vitamin E TPGS, considering the challenges in downstream (drying) process due to possible agglomeration tendency. These studies will focus on the effect of lower concentration of Vitamin E TPGS and optimization of HPMC level on particle size of nanosuspension and also its crystal growth inhibition efficiency during milling and downstream process.

Acknowledgements

The work was carried out within the framework of a research project at Novartis Pharmaceuticals. The authors would like to thank Priya Batheja for her contribution in developing the initial process set-up, Ester Maulit for her help with the HPLC work, Glenn Biank for process support, Hanchen Lee for providing the intralipid suspension and Greg Argentieri for help with the SEM analysis work. Finally the authors would like to thank Ken Yin for conducting the PK study.

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