Original article

Improvement in the dissolution of poorly water soluble drug using media milling technique

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Abstract:

The rate of dissolution is rapidly becoming the leading hurdle in formulation development of poorly water-soluble drugs. In the present work, media milling was employed to formulate nanoparticles of a poorly water-soluble anti-ulcer drug, famotidine (FAM), with the aim of enhancing its dissolution rate. We investigated the nanoparticle formation of FAM via considering the effects of drug-to-stabilizer ratio and amount of beads (zirconium oxide) on the mean particle size, and dissolution properties of FAM. It was observed that optimization of drug-to-stabilizer ratio and amount of beads were allowed the formation of nanosuspensions with a particle size of 244.6 nm. SEM imaging was confirmed the nanosized drug particles. The rate of dissolution of the FAM nanosuspension was enhanced (34.79% in 10 min), relative to that of coarse powder (1.79% in 10 min) and micronized suspension (8.54% in 10 min) mainly due to the formation of nanosized particles.

Keywords: Drug-to-stabilizer ratio; Famotidine; Media milling; Nanosuspension; Zirconium beads

Introduction

In recent years, 40% of the newly developed molecules have poor water solubility problems, which lead to poor bioavailability and high drop out rate from the drug discovery and development from industrial scale. These drugs tend to be eliminated from the gastrointestinal tract before they get the opportunity to fully dissolve and be absorbed into the blood circulation. As about 70% of the human body is made up of water, a drug must be water-soluble and thus possess an acceptable bioavailability level [1, 2].

Many procedures have been investigated to enhance dissolution properties and oral bioavailability of drugs with very low aqueous solubility. Conventional approaches include use of co-solvents, salt formation, pH adjustment, emulsions and micellar dispersions, micronization, complexation with cyclodextrin and solid dispersion. However, most of these techniques require a more amount of additives limiting their use from the safety perspective. Moreover, these techniques offer little help in the formulation of molecules that are poorly soluble in both aqueous and organic solvents [3, 4]. Over the last 10 years, nanoparticle engineering has been developed and reported for pharmaceutical applications. Nanosuspensions are sub-micron colloidal dispersions of solid drug particles in a liquid phase. The different methods used for the preparation of nanosuspensions can be divided into two main categories: top-down approaches, where the raw material is subsequently broken down by using milling methods until nano-sized particles are produced and bottom-up approaches, where nanosuspensions are built up from dissolved drug molecules [5]. The nanosuspension engineering processes currently used are precipitation, pearl milling and high-pressure homogenization, either in water or in mixtures of water and water-miscible liquids or non-aqueous media. The bottom up approaches hold tremendous potential with respect to improving bioavailability in obtaining smaller particle size(<100 nm) and amorphous drug particles, thereby showing similarity to formulation approaches as solid dispersion. However, more work is required before this technique can be commercialized and currently no pharmaceutical application of these systems has been realized. There are some of the drawback of bottom-up processes such as time consuming, scale-up difficulties, low drug loading and wide nanoparticle size distribution. On the other hand, the top-down approach, especially media milling, has been readily accepted by the industry and in fact most of the nanosuspension products currently available on the market are prepared using this technique [3,5,6].

Physical modifications to increase the surface area, solubility and wettability of the drug particles, therefore are focused on particle size reduction or development of amorphous states. Micronization and nanonization are common methods to increase the surface area of the drug. There are several methods for the size reduction of drug particles such as pulverization of large particles using a ball or jet mill. Media milling involves the use of various milling media such as zirconia, and glass balls/beads to reduce the particle size of the compounds and produce sub-micron particle dispersions [5].

Famotidine (FAM) is a histamine H_2-receptor antagonist. It is widely prescribed in gastric ulcers, duodenal ulcers, Zollinger-Ellison syndrome and gastro-esophageal reflux disease. H_2-receptor antagonists, but on equimolar bases, FAM is reported to be about 7.5 and 20 times more potent than ranitidine and cimetidine, respectively, in inhibiting gastric acid secretion. Famotidine has low aqueous solubility (~100 μg/ml) and classified as a BCS class IV substance. Although, FAM undergoes minimal first-pass metabolism and its oral bioavailability has been reported to be low and variable, ranging from 40% to 50% due to its poor aqueous solubility. Since for poorly water-soluble drugs (like famotidine) the dissolution rate is often the rate-limiting step for bioavailability, and the dissolution rate is a function of the solubility and the surface area of the drug, thus dissolution rate will increase if the solubility of the drug is increased, and it will also increase with an increase in the surface area of the drug [7,8].

As the total surface area of the particles in a nanosuspension is typically orders of magnitude larger

compared to a conventional nanosuspension, larger quantities of additives may be required to ensure adequate stabilization. In the absence of a stabilizer, the high surface energy of nanosized particles can induce aggregation, a phenomenon known as Ostwald ripening. Stabilizers need to wet and accumulate at the interface of the drug particles to provide steric or ionic barriers. The type and amount of stabilizer has a pronounced effect on the physical stability of nanosuspensions. A mixture of stabilizers is sometimes required to obtain a stable nanosuspension. Stabilizers that have been explored so far include cellulose derivatives, poloxamers, polysorbates, lecithin and povidones. Therefore, whatever method used for the production of nanosuspensions, a careful evaluation of the type and amount of the stabilizer used is key to the successful production of nanosuspensions. Both polymeric stabilizers and surfactant stabilizers can be used for this purpose [3,6,9].

In this work the production of nanosuspensions was intended for oral use of FAM using a media milling technique at laboratory scale. The aim of this investigation was to ascertain the role of drug-to-stabilizer ratio and amount of grinding media for preparation of FAM nanosuspension. To this purpose several nanosuspensions were prepared using polymeric stabilizers and surfactant stabilizers with varying amount of grinding media. For polymeric stabilizers, polyvinyl pyrrolidone (PVPK-30) and hydroxypropylmethylcellulose (HPMCK15M) were tested. For non-ionic surfactant stabilizers, Pluronic-F68, Pluronic-F127 and Tween-80 were tested. Tween-80 and PVPK-30 were employed as combination of non-ionic surfactant stabilizer with polymeric stabilizer respectively in terms of enhancing stabilization of nanosuspension. An attempt to establish the relationship among the grinding media and stabilizers was made by analyzing the results of mean particle size. Characterization of nanosuspensions was carried out by different techniques, particle size analysis, dissolution test, and scanning electron microscopy (SEM) imaging. Dissolution study of nanosuspension formulations was performed in 0.1 N HCL (pH 1.2) and was compared to that of micronized suspension and coarse powder of the drug sample.

Material and Methods

Materials

FAM was obtained as a gift sample from Cadila Pharmaceutical Ltd., India. Pluronic-F68 and Pluronic-F127 were obtained as gift samples from Torrent Pharmaceutical Ltd., India. Hydroxypropylmethylcellulose (HPMCK15M) was obtained as a gift sample from Colorcon Asia Ltd., India. Polyvinylpyrrolidone (PVPK-30) and Tween-80 were gifted from Sisco Research Laboratory Pvt. Ltd. India and S.D Fine Chemical Ltd., India, respectively. Double distilled water was used in the study. All materials used were conformed to USP 24 standards.

Preparation of micronized suspension

Micronized suspension of the drug was prepared by dispersing FAM in a Tween-80-PVPK-30 bidistilled water solution using magnetic stirring at 250 rpm (Remi, India) at room temperature for 24 h. Micronized suspension was prepared using 1:2 drug-to-surfactant ratio (w/w).

Preparation of nanosuspensions

Nanosuspensions were prepared using media milling. In 20 ml vials, suspensions of 40 mg FAM in 10 ml water were prepared. Two sets of zirconium beads (ø 0.5 mm) as a milling agent with drug-to-stabilizer ratio of 1:1 and 1:2 (w/w) were used. First set of 6 gm zirconium beads with drug-to-stabilizer ratio of 1:1 and 1:2 (Pluronic-F68, Pluronic-F127, PVPK-30, HPMCK15M and combination of Tween-80-PVPK-30) and second set of 8 gm zirconium beads with same drug-to-stabilizer ratios were prepared. The comminuting process was performed on a high-speed shaker (Remi, India) at 250 rpm at room temperature for 24 h. After milling, nanosuspensions were separated from the zirconium beads by decanting the suspension followed by washing of the beads with bidistilled water. In total, 20 vials of nanosuspension were prepared (Table 1). Milling was carried out under ambient conditions for throughout experiment.

Table 1 Composition of famotidine nanosuspensions

Formulations	Stabilizers	Drug-to-stabilizer ratio	Amount of zirconium beads (gm)
W_1	Pluronic -F127	1:1	6
W_2	Pluronic -F127	1:1	8
W_3	Pluronic -F127	1:2	6
W_4	Pluronic -F127	1:2	8
W_5	Pluronic-F68	1:1	6
W_6	Pluronic-F68	1:1	8
W_7	Pluronic-F68	1:2	6
W_8	Pluronic-F68	1:2	8
W_9	PVPK-30	1:1	6
W_{10}	PVPK-30	1:1	8
W_{11}	PVPK-30	1:2	6
W_{12}	PVPK-30	1:2	8
W_{13}	HPMCK15M	1:1	6
W_{14}	HPMCK15M	1:1	8
W_{15}	HPMCK15M	1:2	6
W_{16}	HPMCK15M	1:2	8
W_{17}	Tween-80-PVPK-30	1:1	6
W_{18}	Tween-80-PVPK-30	1:1	8
W_{19}	Tween-80-PVPK-30	1:2	6
W_{20}	Tween-80-PVPK-30	1:2	8

Note: Drug-to-stabilizer ratio was changed respect to 40 mg famotidine in media milling technique

Physical appearance

To evaluate the physical appearance, nanosuspension samples were observed for agglomeration and color change for two weeks at room temperature after media milling and pictures were obtained using a Kodak C-140 camera (Eastman Kodak Company. NY).

Particle size analysis

Particle size analysis of the nanosuspension formulations was performed by photon correlation spectroscopy (PCS) using a Zetasizer 3000 (Malvern Instruments, UK). This technique yielded the mean particle diameter. The formulations were added drop-wise to the sample dispersion unit containing water (saturated with FAM) as a dispersant. A refractive index value of 1.5 was used for particle size analysis. All the data presented are the mean values of the results on three independent samples produced under identical conditions.

Scanning electron microscopy

The surface morphology of the micronized powder and formulated nanosuspension were visualized by scanning electron microscopy (SEM). Particle morphology was investigated using a Hitachi (S-4700, Japan) scanning electron microscope with an acceleration voltage of 30 kV. Samples were prepared by drying suspension droplets on clean SEM sample stages, followed by coating with Pt-Pd for 2 min.

Drug release studies from nanosuspension

A double jacketed beaker was used as a dialysis system to study the dissolution of FAM from nanosuspensions (Figure 1). The drug release from nanosuspension was determined using a dialysis tube (donor compartment) containing the known quantity (10 ml) of the nanosuspension in a water-jacketed beaker containing 300 ml of 0.1 N HCL (pH 1.2) at 37 $\pm$ 1°C. The contents of the

beaker were agitated on a magnetic stirrer. Five milliliters of sample was withdrawn periodically (after 10 min) and replaced with an equal volume of fresh 0.1 N HCL (pH 1.2) up to 120 min. Samples were diluted suitably and filtered through a filter paper (0.22 µm, Whatman Inc., USA). The dialyzate was then subject to the UV analysis against the blank (0.1 N HCL solution). Percent cumulative release of FAM was calculated based on the standard UV calibration curve at 267 nm (Systronic 2203, Japan). It was noted that there was no any interference of stabilizer released into the dissolution medium. For comparison, a suspension of micronized FAM with the same amount of Tween-80-PVP-K30 was used. The dissolution rate of pure FAM in powder form was also measured.

Results

Media milling with zirconium beads has been employed to produce nanosuspension of FAM. In media milling process, Pluronic-F68, Pluronic-F127, PVPK-30, HPMCK15M and combination of Tween-80-PVPK-30 were used as stabilizers. We investigated extensively the milling process variables and conditions such as milling time, milling speed, drug-to-stabilizer ratio, amount of grinding media, and volume of media. From these formulative variables, drug-to-stabilizer ratio and amount of grinding media (zirconium beads) were contributing much towards the change in particle size in nanosuspension preparation.

Physical appearance

Visually identification of nanosuspension was done on a basis of color change and agglomeration. There was not found any color change after preparation of nanosuspensions. Sedimentation of Pluronic-F68, Pluronic-F127, and HPMCK15M contained nanosuspension occurred within 6-7 h. Although the nanosuspension of the PVPK-30 was stable at room temperature for at least two days, we found aggregated nanoparticles after two days. However, significantly higher particle sizes were obtained in the case of suspensions made with Pluronic-F68, Pluronic-F127, and HPMCK15M. PVPK-30 alone did not demonstrate any significant effect on stabilization of nanoparticles and yield larger particle size after two days. We found that Tween-80 improved stabilization in respect to rigid molecular interactions with PVPK-30 in the suspension even after two weeks (Figures 2(A) and 2(B)). It was expected to contribute to the stabilization of nanoparticles through adsorption of PVPK-30 and Tween-80 onto the drug nanoparticles surface in the suspension.

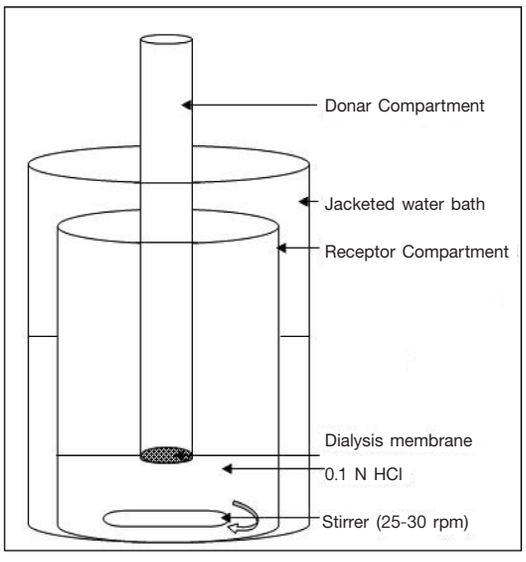

Figure 1 Schematic representation of modified diffusion cell apparatus

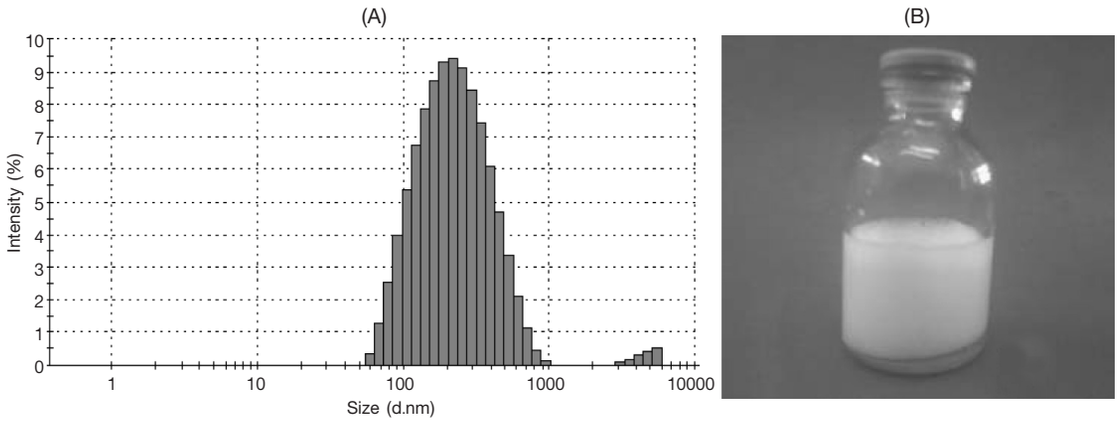

Figure 2 (A) Particle size distribution of FAM nanosuspension (W_{20}). (B) Appearance of FAM nanosuspension (W_{20}) at two weeks after preparation.

Influence of stabilizers and zirconium beads

For the effective size reduction of the drug particles, water soluble polymers and surfactants have been used as additives to inhibit the particles agglomeration and improve the physicochemical properties of the drug. Influence of different stabilizers was investigated in media milling method with a fixed concentration of the drug. The type of compound and their amount employed for stabilization has a pronounced effect on particle size. Small particles, which spontaneously aggregate to decrease the surface energy, were stabilized by a layer of surfactant or/and protective polymer. Four stabilizers (Pluronic-F68, Pluronic-F127, PVPK-30 and HPMCK15M) were tested for their stabilization potential. Important function of polymers is that they can form a substantial mechanical and thermodynamic barrier at the interface that retards the approach and coalescence of individual nanoparticles. When we evaluated feasibility of nanosuspension production, poor performance was seen when the stabilizers were Pluronic-F68, Pluronic-F127, and HPMCK15M. For Pluronic-F68 and Pluronic-F127, increasing drug-to-stabilizer ratio did not result in stabilization of nanosuspension. It was noticed that when drug-to-stabilizer ratio was shifted from 1:1 to 1:2 for HPMCK15M, it produced more viscosity compared to other stabilizers. More viscosity of HPMCK15M was inherent disadvantage as it was slow down the process for nanosuspension. For PVP-K30, increasing drug-to-stabilizer ratio from 1:1 to 1:2 markedly improved stabilization of nanosuspension. The mechanism of the adsorption of PVPK-30 is likely by the formation of steric barriers. This effect was more pronounce with PVPK-30, having shortest chain length. Steric barriers are produced when the adsorbed polymer extends its chain to the water phase, which helps maintaining the distance between closely approaching solid particles. In case of Tween-80, it could enhance the repulsion and dispersion of the dispersed particles in the aqueous system. It was noticed that effect of Tween-80-PVPK-30 was more significant compared to PVPK-30 in terms of stabilization of nanosuspension. It can be seen that drug-to-stabilizer at a ratio of 1:2 for Tween-80-PVPK-30 was likely to give enough electrostatic repulsion and steric barrier to prevent aggregation.

It was found that not only use of change in ratio of Tween-80-PVPK-30 respect to that of drug, but also amount of zirconium beads played significant role in stabilization of nanosuspension. Eight gm of zirconium beads and drug-to stabilizer in a ratio of 1:2 (W_{20}) yielded smaller particle size (244.6 nm) than that with drug-to stabilizer at a ratio of 1:1 (W_{18}). On other side of media milling, drug suspension at 6 gm of zirconium beads led to larger particle size irrespective of drug-to stabilizer at ratios of 1:1 or 1:2 (W_{17} and W_{19}) (Figure 3). One possible explanation for this is that there is contribution of small gap in presence of 8 gm of zirconium beads bed when suspension undergoes intense media grinding. More quantity of zirconium beads has the less void, leads to smaller particle size. These induced cracks the particles by a combination of impact and attrition

induced by rotating the beads at 250 rpm on high speed shakers. The freshly created particulate surfaces were immediately coated by a layer of Tween-80-PVPK-30 (especially for drug-to stabilizer in 1:2) using 8 gm of zirconium beads, which prevented the broken particles from agglomerating.

Scanning electron microscopy

Figure 4 shows an SEM micrograph of the micronized FAM powder particles and image of the FAM nanosuspension. Micronized FAM powder showed irregular shapes with particle size generally larger than 5 µm. Particles generated by media milling were primarily spherical with diameters slightly less than 500 nm obtained by PCS.

Drug release studies from nanosuspension

The *in vitro* dissolution of a drug is an indirect method to predict its bioavailability from a formulation. Since nanosuspension W_{20} had shown the highest stability against aggregation, dissolution behaviour was investigated only for this formulation. $DP_{10\ min}$ (percent drug dissolved within 10 minutes), mean dissolution time (MDT) and $t_{50\%}$ (time to dissolve 50% drug) values

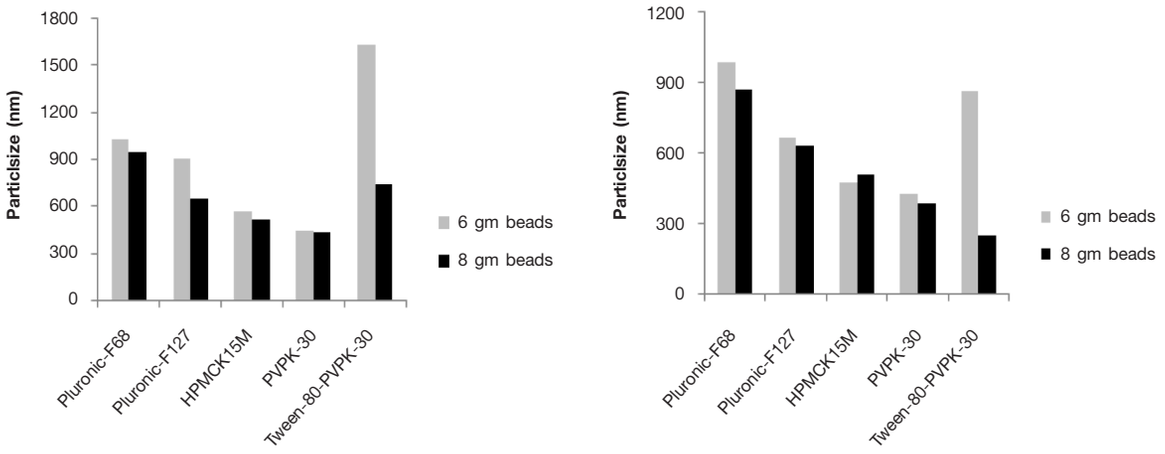

Figure 3 Mean particle size of FAM nanosuspensions obtained in drug-to stabilizer at a ratio of 1:1 (left) and mean particle size of FAM nanosuspensions obtained in drug-to stabilizer at a ratio of 1:2 (right)

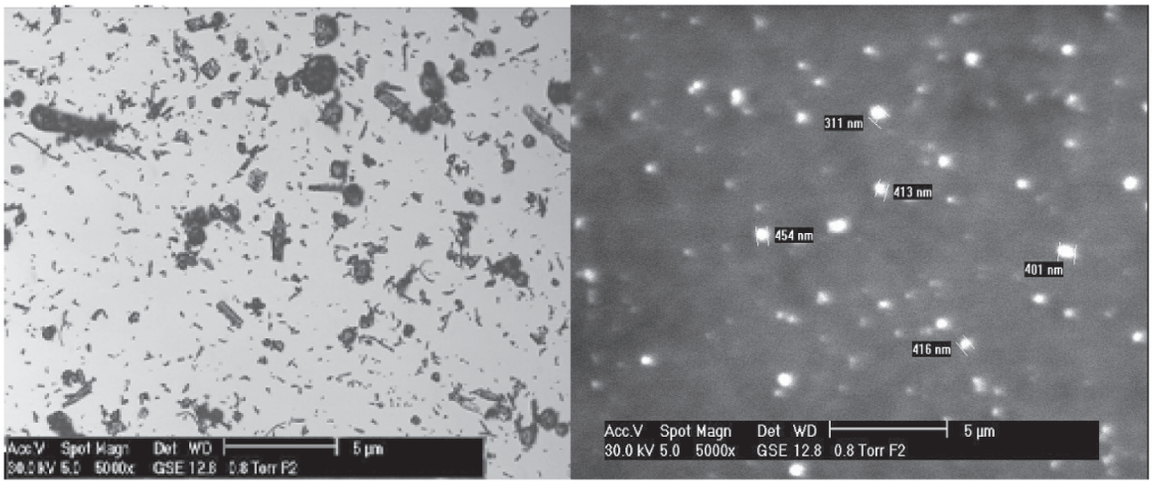

Figure 4 SEM images of micronized powder of FAM (left) and FAM nanosuspension after medial milling (right)

calculated from release profile are reported in Table 2 for coarse powder, micronized suspension and nanosuspension. From this data, it was evident that onset of dissolution of pure FAM was very low ($DP_{10\ min}$ value 1.79% and $t_{50\%}$ >>2 h).

As shown in Figure 5, nano-sized FAM showed a dramatic increase of rate and extent of dissolution compared to micronized FAM with the same amount of Tween-80-PVPK-30 and much larger compared to powder form of FAM. The slope of dissolution profile is especially different for nanoparticles in the initial stage (first 10min) and maintained throughout the experiment compared to micronized FAM. Drug nanoparticles of FAM were shown higher surface area compared to micronized particles in suspension and coarse particles in powder.

MDT reflects the time for the drug to dissolve and is the first statistical moment for the cumulative dissolution process that provides an accurate drug release rate [10]. Since it is accurate expression for drug release rate, a lower MDT value indicates faster dissolution rate [11].

$$MDT_{in\ vitro} = \frac{\sum_{i=1}^{n} t_{mid}\,\Delta M}{\sum_{i=1}^{n} \Delta M} \quad (1)$$

where t_{mid} is the time at midpoint and ΔM is the additional amount of drug dissolved in the period of time. MDT value of pure FAM is very high (37.95 min) and the value was decreased to a greater extent after preparing in nanosuspension. Nanosuspension showed lowest MDT value of 19.37 min. MDT was considerably lower for the nanosuspension formulation, indicating a faster dissolution rate compared to the coarse powder, and micronized suspension (Table 2).

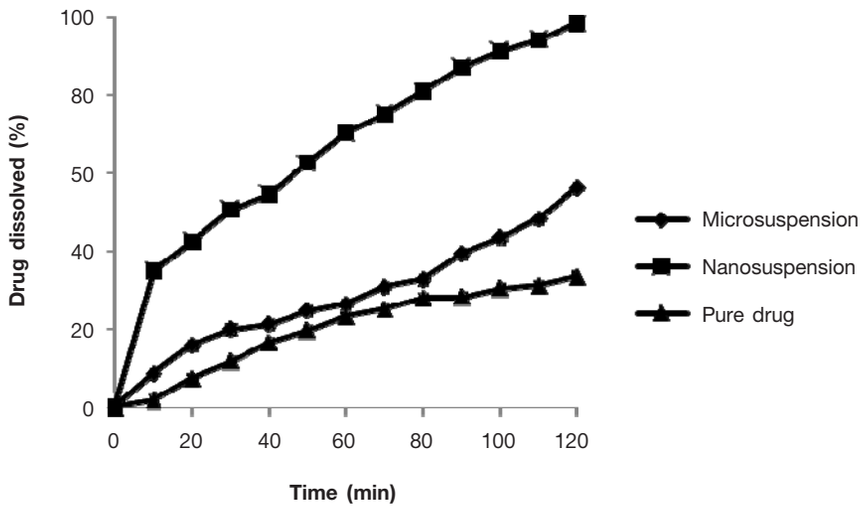

Figure 5 The dissolution profiles of nano-sized FAM stabilized with Tween-80-PVPK-30 using 8 gm zirconium beads, the suspension of micronized FAM with the same amount of Tween-80-PVPK-30 and the pure FAM. Data points are means ?SD for separate triplicate samples

Table 2 % Drug dissolved within 10 minutes (DP_{10min}), time to dissolve 50% drug ($t_{50\%}$) and mean dissolution time (MDT) of FAM in powder, microsuspension and nanosuspension

Dissolution	Coarse powder	Microsuspension	Nanosuspension
DP_{10min} (min)	1.79	8.54	34.79
MDT (min)	37.95	25.71	19.37
$T_{50\%}$ (min)	>120	110	30

In addition, the time to dissolve 50% of the drug ($t_{50\%}$) was strongly reduced in nanosuspension. As can be seen, for each formulation DP_{10min} values increased in the following order: nanosuspension<micronized suspension<coarse powder; while MDT and $t_{50\%}$ values decreased in the same order. Moreover, all dissolution parameters demonstrated that nanosuspension (W_{20}) had better dissolution properties than micronized suspension and coarse powder.

Discussion

The milling process used for the production of drug nanoparticles can be described as a simple procedure comprising attrition media, suspension and agitation. The high energy and shear forces generated as a result of the impaction of the milling media with drug particles provides energy for size reduction of drug molecules into nanoparticles. Nanosuspensions have the drawback of instability caused by nucleation and particle growth. In the absence of stabilizers or surfactants, the high surface energy of nano-sized particles can induce aggregation-a phenomenon known as Ostwald ripening. The use of surfactant/polymer mixtures is often considered to have synergistic effects in formation of nanosuspension. Stabilizers or surfactant are accumulating at the interface of the drug particles to provide steric or ionic barriers. The type and amount of stabilizer has a pronounced effect on the physical stability nanosuspensions. Depending on the type of polymers and surfactants, either attractive or repulsive interactions between the polymers and surfactants can occur. The effectiveness of polymeric materials such as Pluronic-F68, Pluronic-F127, PVPK-30, and HPMCK15M is significantly smaller than combination of Tween-80-PVPK-30 in terms of particle size. Since, PVPK-30 was chosen as polymeric stabilizer to prevent aggregation of nano-sized particles, and the rationale for using combination of Tween-80-PVPK-30 was based on combined electrostatic and steric stabilization. The combination of Tween-80 and PVPK-30 was used for stabilization of nanosuspensions because it was examined for drug/Tween-80/PVPK-30 ternary ground mixtures.

It was observed that the extent of size reduction is mainly governed by the amount of zirconium beads in media milling in our experiment. As the zirconium beads rotated, they flew through the grinding jar interior and impact against the sample on the opposite grinding jar wall. The combination of frictional forces and impact forces produce a high degree of particle size reduction. It was found that vials contained beads that occupied 30% and 40% of mill volume respected to 6 gm and 8 gm of beads, respectively. In the present study, it can be observed that at less amount of beads (6 gm) generated the energy imparted to the system and was not enough to cause complete particle size reduction. Instead of that, activated regions were generated which may be responsible for agglomeration and hence particle size was increased on storage. At high amount of beads (8 gm) the energy imparted to the system was sufficient to break down the particles completely. Accordingly, there was a lower tendency for the generation of activated regions and therefore these suspensions were stable with respect to particle size. In our study, media milling of drug suspension at 6 gm of zirconium beads (30%) led to larger particle size than 8 gm of zirconium beads (40%). The void among bead is a function of the occupied volume by beads, which means it decreases with increasing amount of beads. Occupied volume of beads is directly related to particle size reduction. Based on increasing amount of beads, it was lead to efficient particle size reduction due to fewer voids among beads.

In vitro dissolution screening should be the first line of biopharmaceutical evaluation of nanosuspensions. It was observed that dissolution of micronized FAM was more compared to coarse powder. The slow dissolution can be partly attributed to its hydrophobicity as evidenced by poor wetting of the powder surface. This causes the particles to aggregate rather than to disperse. The improvement of dissolution rate in nanosuspension is possibly caused by increased surface area, which enhances strong hydrophilic character of drug toward PVPK-30 *via* formation of intermolecular hydrogen bonds and improved wettability of hydrophobic FAM using Tween-80. An improvement in dissolution rate of poorly water soluble drug was more effective *via* particle size reduction (nanosuspension) rather than

use of mixing or blending of polymers or surfactants (micronized suspension).

Conclusion

Media milling technique has been described as a simple method for drug nano-sizing at laboratory scale. Particle size is influenced by modifying the drug-to-stabilizer ratio and amount of zirconium beads. Nanosized FAM dissolved significantly faster than micronized drug suspension and raw drug powder. Moreover, the fact that the micronized suspension of the drug and stabilizer did not significantly improve the dissolution of the drug suggesting that the increased dissolution rate for the nanosuspension is primarily governed by reduction of the particle size. These findings indicate the suitability of formulation procedure for preparation of nanosized poorly water-soluble drug with significantly improved *in vitro* dissolution rate, and thus possibly enhancing fast onset of therapeutic drug effect.

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