ORIGINAL ARTICLE

Formulation, optimization and characterization of gemfibrozil nanocrystals prepared by wet milling technique

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Today, nanotechnology has a variety of application areas. Pharmacy is one of the most important application fields of nanotechnology. Preparation of nanoparticular drug delivery systems such as nanocrystals could improve the solubility and bioavailability of poorly water soluble drugs. Gemfibrozil (GEM) is a low water soluble drug biopharmaceutical classification system II and used as a lipid regulating agent. In this study, a rapid and simple wet milling method was used for preparation of GEM nanosuspension (GEM NS). The use of sonication after wet milling process reduced the milling time significantly. Different concentrations of stabilizers (polyvinyl pyrrolidone K30 [PVP K30] and Tween 80) were tested for preparation of GEM NSs. The finest GEM NS was obtained by 0.5% w/v GEM, 1% w/v PVP K30 and 2% w/v Tween 80. The size and zeta potential of finest GEM NS were 238.2 ± 2.5 nm and - 19.6 ± 0.1 mV, respectively. The morphology of dried GEM NS was observed using atomic force microscopy. Differential scanning calorimetry of GEM and GEM NS confirmed that there was no interaction between GEM and stabilizers. Compared with GEM, the solubility of GEM NS increased significantly.

Key words: *Gmefibrozil, nanocrystal, poorly soluble drugs, wet milling*

INTRODUCTION

Approximately, 40% of newly discovered drugs show poor solubility in water.[1] The poor water solubility of drugs causes their poor bioavailability.[2] There are many approaches for improving the water solubility of poor water soluble drugs. Some of these approaches include salt formation of drugs, use of co-solvents, surfactants and complexing agents.[3] Moreover, it has been reported that particle size reduction of drugs could increase the solubility of drugs.[4] Today, nanotechnology is an important tool to increase the solubility of poor water soluble drugs. The particle size reduction of these drugs to the nanometer range could improve their dissolution rate and bioavailability because of increased their surface area and saturation solubility.[5,6] Wet milling is an efficient technique used to reduce particle size of drugs to nanometer range and increase their water solubility.[7] In this technique, the drug particles in aqueous suspension are size-reduced by grinding using small and hard beads. The drug particle size is reduced by the attrition of drug particles with the grinding media.[8] Nano-sized particles tend to form aggregates as a result of their large surface area.[9] In order to minimize drug particle aggregation, it is necessary to stabilize drug particles using stabilizers such as polymers and surfactants.[10] The stabilizers adsorb on the surface of drug particles and stabilize the drug particles through steric or ionic interactions.[11] The main drawback of wet milling technique is that processing time may be long.[12,13] Gemfibrozil (GEM) is a lipid regulating agent that decreases serum triglycerides and very low density lipoprotein cholesterol and increases high-density lipoprotein (HDL) cholesterol.[14] Studies show that, GEM could prevent major cardiovascular events by increasing HDL-cholesterol.[15] According to the Biopharmaceutical

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Classification System, GEM is classified under class-II drugs.[16] Class-II drugs are the drugs with poor solubility and high permeation in the human body and pose problems in their pharmaceutical product development process.[17] The aim of this work is to prepare GEM nanosuspensions (GEM NS) using a modified wet milling method to increase its water solubility. In this work, we used a combination of sonication and wet milling method for the preparation of GEM NS. This combination reduced the processing time of wet milling. The optimized NS of GEM was characterized by particle size and size distribution, atomic force microscopy (AFM), differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy.

MATERIALS AND METHODS

Materials

Gemfibrozil and Tween 80 (polyoxyethylene sorbitan monooleate) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Polyvinyl pyrrolidone K30 (PVP K30) was purchased from Fluka (Germany). All other materials used were of analytical grade.

Preparation of gemfibrozil nanosuspension

For the preparation of stabilizer solution, Tween 80 was dissolved in purified water, and then PVP K30 was added under mechanical agitation. Pure GEM was dispersed in the stabilizer solution and kept under mechanical agitation. Once a uniform suspension was formed, the suspension was placed in a glass tube. Glass beads (2 mm in diameter) or grinding media were placed in a glass tube. NS was prepared after milling the suspension using glass beads. The ratio between the suspension and the grinding media was (1:1, v/v). Tubes were shaken using an IKA® orbital shaker (Vibrax VXR Basic, Germany) with 1500 rpm at room temperature for 75 min. Then, milled suspension was sonicated using an ultrasonic bath (Eurosonic 4D, Italy) at 37°C for 30 min. Different concentrations of stabilizers were tested for preparation of the finest GEM NS. The details of the formulation design are described in Table 1.

Particle size and zeta potential analysis

Ten microliter of NS was diluted with 1 ml purified water. Then the size, polydispersity and zeta potential of the NSs were determined by Malvern Zetasizer Nano ZS 90 (Malvern Instruments Ltd., Worcestershire, UK).

Table 1: Formulation design of GEM NS using different concentration of stabilizers

Formulation	GEM %	PVP K30%	Tween 80%	Mean particle size	PDI
F1	0.5	2	1	435.4±3.1	0.432±0.04
F2	0.5	1	2	238.2±2.5	0.465±0.05
F3	0.5	2	2	484.8±9.9	0.5±0.02

PDI: Poly dispersity index, PVP K30: Polyvinyl pyrrolidone K30, GEM NS: Gemfibrozil nanosuspensions

Differential scanning calorimetry

Thermal properties of GEM and dried GEM NS were analyzed using a DSC-1 (Mettler Toledo, Switzerland). Approximately, 5 mg of samples were placed in aluminum pans. The measurements were carried out at temperatures from 20°C to 250°C at a scan rate of 20°C/min.

Atomic force microscopy

The AFM analysis was carried out to characterize the morphology (dimensional image) using a NanoWizard system (JPK Instruments AG, Berlin, Germany). A drop of diluted NS was placed on a mica surface and allowed to dry at room temperature. AFM imaging was performed in tapping mode in air at room temperature using a silicon nitride cantilever with a spring constant of 40 Nm^{-1}.

Fourier transform infrared

Dried GEM NS, GEM and PVP K30 were diluted with potassium bromide and made into pellets. The FTIR spectra of pure GEM, PVP K30 and dried GEM NS were recorded using an FTIR spectrometer (Bruker, Tensor 27, Ettlingen, Germany).

Determination of solubility for gemfibrozil (GEM) and GEM nanosuspensions

The aqueous solubility of GEM and GEM NS were determined by a shake-flask method. Briefly, an excess amount of GEM and dried GEM NS were suspended in 9 ml of phosphate buffer pH 7.5, and the suspensions were shaken at 37°C. Aliquots of solutions were withdrawn and filtered through a 0.22 μm Whatman filter. The concentration of GEM was determined in filtrates using Thermo UV/Visible Spectrophotometer (Genesys, USA) at 276 nm.

RESULTS

As shown in Table 1, several formulations of GEM NS were prepared using different concentration of Tween 80 and PVP K30 as stabilizers. Drug concentration was fixed at 0.5%. Among the different concentration of stabilizers, the finest GEM NS (F2) was obtained by 1% w/v PVP K30 and 2% w/v Tween 80. Particle size, zeta potential and poly dispersity index (PDI) of GEM NSs, were determined using laser light scattering. Particle size, PDI and zeta potential of F2 were 238.2 ± 2.5 nm, 0.465 ± 0.05 and − 19.6 ± 0.1 mV respectively.

Atomic force microscopy

The AFM image of the finest GEM NS (F2) is shown in Figure 1. The particles of the finest GEM NS (F2) were approximately homogenous and spherical in shape. The dimension of these nanoparticles was 253 ± 40 nm in length.

Differential scanning calorimetry

Figures 2 and 3 show DSC thermograph of pure GEM and the finest GEM NS (F2) respectively. Pure GEM showed a

typical endothermic peak at 60.91°C, which corresponds to its melting point and the finest GEM NS (F2) showed an endothermic peak at 55.8°C.

Fourier transform infrared

The molecular states of GEM and the finest GEM NS (F2) were investigated using FTIR. Figure 4 shows the FTIR spectrum of the GEM and the dried finest GEM NS (F2) in the range of 400-4000 cm^{-1}. The spectrum of the dried finest GEM NS (F2) showed no obvious difference with the GEM spectra in the whole area of GEM absorption bands.

Solubility for gemfibrozil (GEM) and GEM nanosuspensions

The solubility of GEM in buffer was 1.0 ± 0.01 μg/ml while the solubility of GEM from the dried finest GEM NS (F2) was 8.2 ± 0.03 μg/ml. Therefore, the solubility of GEM from the dried finest GEM NS (F2) was almost 8-times higher than the parent GEM.

DISSCUSION

Recently, wet milling technology is successfully used for formulating of poorly water soluble drugs. Nanosized drug particles prepared by wet milling technique could improve the solubility and bioavailability of these drugs.[8,18,19] However, the main drawback of this technique is that processing time may be long.[12,13] Many researchers have attempted to reduce the processing time by different strategies.[13,20] For example, drug particle size reduction using a jet mill before wet milling process could reduce the processing time of wet milling.[20] Also, new combinational methods have been developed for particle size reduction. These combinational methods could overcome the limitation of conventional size particle size reduction technologies and reduce the drug nanocrystal production time. Salazar *et al*.[13] produced glibenclamide nanocrystals using a combination of a nonaqueous freeze drying followed by wet ball milling or high-pressure homogenization. They could reduce the milling time from 24 h to 1 h. In this study, we used sonication to reduce the processing time of wet milling for preparation of GEM NS. Already, we could prepared GEM NS (mean particle size: 245 ± 12 nm) by wet milling method using Tween 80 and PVP K30 as stabilizers after 24 h milling time. In this study, the use of sonication after wet milling process reduced the milling time from 24 h to 75 min. As shown in Table 1, the finest GEM NS (F2) showed mean particle size of 238.2 ± 2.5 nm with PDI of 0.465 ± 0.05. The zeta potential of the finest GEM NS (F2) was around - 20 mV, which is enough for sufficient electrostatic stabilization of GEM NS.[21] The morphology and size of the finest GEM NS (F2) were evaluated by AFM. The data obtained by AFM also confirmed DLS analysis results. We

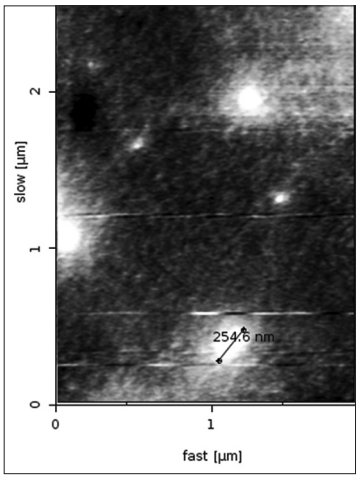

Figure 1: Atomic force microscopy image of the finest gemfibrozil (GEM) nanosuspensions (F2). The concentrations of GEM, Tween 80 and polyvinyl pyrrolidone K30 were 0.5%, 2% and 1% respectively

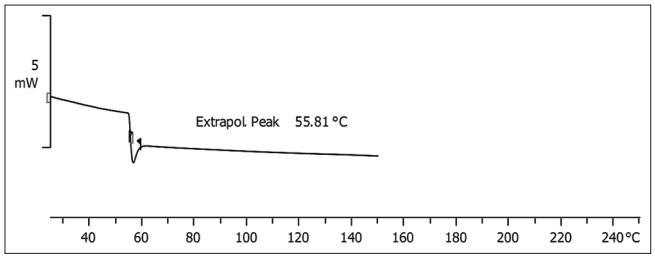

Figure 3: Differential scanning calorimetry thermogram of the finest gemfibrozil (GEM) nanosuspensions (F2). The concentrations of GEM, Tween 80 and polyvinyl pyrrolidone K30 were 0.5%, 2% and 1%

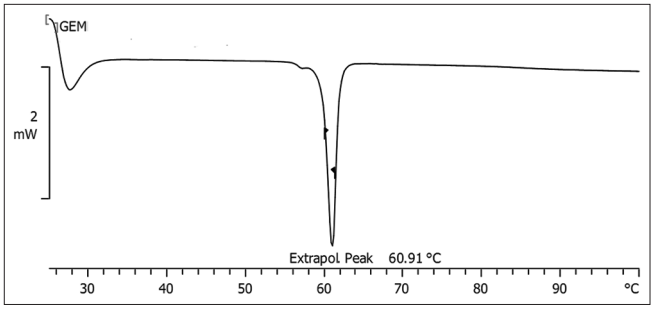

Figure 2: Differential scanning calorimetry thermogram of bulk gemfibrozil powder

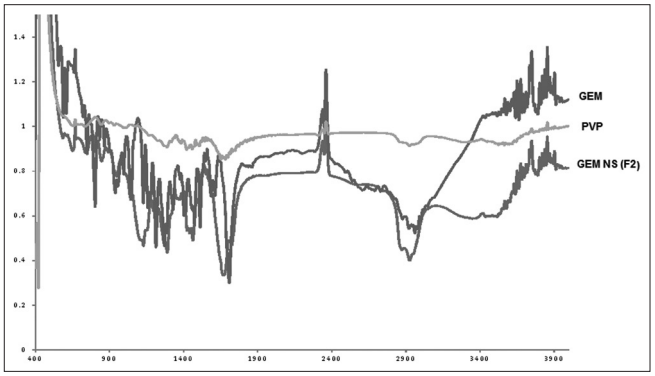

Figure 4: Fourier transform infrared spectra of gemfibrozil (GEM), polyvinyl pyrrolidone (PVP) K30 (PVP) and the finest GEM nanosuspensions (F2)

used DSC technique to investigate the effect of stabilizers on the GEM structure. Comparing the DSC thermograms of GEM and GEM NS, no significant differences were found between GEM and the finest GEM NS (F2) curves. It was concluded that there was no interaction between GEM and stabilizers. The shift of GEM peak in the GEM NS sample to the lower temperature could be due to the presence of Tween 80 on the surface of GEM nanocrystals.[22] The FTIR spectra of pure GEM and GEM NS confirmed the presence of GEM in the GEM NS. Moreover from the results of FTIR study, it can be concluded that there is no evidence of chemical interaction between GEM and stabilizers. During solubility study, it was observed that the solubility GEM NS has been increased up to 8-fold due to the formation of GEM nanocrystals.

CONCLUSION

In this study, NS of a poorly soluble drugs, GEM, was prepared easily using a combination of wet milling method and sonication. This NS could be used for preparation of a promising new drug formulation of GEM. Solubility study in water shows that GEM NS gives higher GEM solubility compared to the pure GEM. Consequently, GEM NS could represent a promising alternative drug delivery system to improve the bioavailability of GEM.

REFERENCES

1. Lipinski C. Poor aqueous solubility - an industry wide problem in drug discovery. Am Pharm Rev 2002;5:82-5.
2. Muller RH, Peters K. Nanosuspensions for the formulation of poorly soluble drugs: I. Preparation by a size reduction technique. Int J Pharm 1998;160:229-37.
3. Chaudhary A, Nagaich U, Gulati N, Sharma VK, Khosa RL. Enhancement of solubilization and bioavailability of poorly soluble drugs by physical and chemical modifications: A recent review. J Adv Pharm Edu Res 2012;2:32-67.
4. Jinno J, Kamada N, Miyake M, Yamada K, Mukai T, Odomi M, *et al.* Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs. J Control Release 2006;111:56-64.
5. Bhalekar MR, Upadhaya PG, Reddy S, Kshirsagar SJ, Madgulkar AR. Formulation and evaluation of acyclovir nanosuspension for enhancement of oral bioavailability. Asian J Pharm 2014;8:110-8.
6. Mokale V, Patil K, Khatik T, Sutar Y. Glyburide nanosuspension: Influence of processing and formulation parameter on solubility and *in vitro* dissolution behavior. Asian J Pharm 2013;7:111-7.
7. Inkyo M, Tahara T. Dispersion of agglomerated nanoparticles by fine Beads mill. J Soc Powder Technol Jpn 2004;41:578-85.
8. Van Eerdenbrugh B, Van den Mooter G, Augustijns P. Top-down production of drug nanocrystals: Nanosuspension stabilization, miniaturization and transformation into solid products. Int J Pharm 2008;364:64-75.
9. Peukert W, Schwarzer H, Stenger F. Control of aggregation in production and handling of nanoparticles. Chem Eng Process 2005;44:245-52.
10. Van Eerdenbrugh B, Vermant J, Martens JA, Froyen L, Van Humbeeck J, Augustijns P, *et al.* A screening study of surface stabilization during the production of drug nanocrystals. J Pharm Sci 2009;98:2091-103.
11. Ploehn HJ, Russel WB. Interactions between colloidal particles and soluble polymers. Adv Chem Eng 1990;15:137-228.
12. Chan HK, Chew NY. Novel alternative methods for the delivery of drugs for the treatment of asthma. Adv Drug Deliv Rev 2003;55:793-805.
13. Salazar J, Müller RH, Möschwitzer JP. Application of the combinative particle size reduction technology H 42 to produce fast dissolving glibenclamide tablets. Eur J Pharm Sci 2013;49:565-577.
14. Kersten S, Mandard S, Tan NS, Escher P, Metzger D, Chambon P, *et al.* Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene. J Biol Chem 2000;275:28488-93.
15. Rubins HB, Robins SJ, Collins D, Fye CL, Anderson JW, Elam MB, *et al.* Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans affairs high-density lipoprotein cholesterol intervention trial study group. N Engl J Med 1999;341:410-8.
16. Bergman E, Matsson EM, Hedeland M, Bondesson U, Knutson L, Lennernäs H. Effect of a single gemfibrozil dose on the pharmacokinetics of rosuvastatin in bile and plasma in healthy volunteers. J Clin Pharmacol 2010;50:1039-49.
17. Amidon GL, Lennernäs H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: The correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. Pharm Res 1995;12:413-20.
18. Rogers TL, Gillespie IB, Hitt JE, Fransen KL, Crowl CA, Tucker CJ, *et al.* Development and characterization of a scalable controlled precipitation process to enhance the dissolution of poorly water-soluble drugs. Pharm Res 2004;21:2048-57.
19. Mittapalli PK, Yamasani MR, Shashank A. Improved bioavailability of albendazole following oral administration of nanosuspension in rats. Curr Nanosci 2007;3:191-4.
20. Möschwitzer J, Müller RH. New method for the effective production of ultrafine drug nanocrystals. J Nanosci Nanotechnol 2006;6:3145-53.
21. Müller RH, Jacobs C, Kayser O. Nanosuspensions as particulate drug formulations in therapy. Rationale for development and what we can expect for the future. Adv Drug Deliv Rev 2001;47:3-19.
22. Teeranachaideekul V, Junyaprasert VB, Souto EB, Müller RH. Development of ascorbyl palmitate nanocrystals applying the nanosuspension technology. Int J Pharm 2008;354:227-34.

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