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Research Article

Polysorbate 80 – Modulated nanocrystallization for enhancement of griseofulvin solubility

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Abstract

Griseofulvin (GRFV1) is used in the treatment of many fungal infections, including ringworm and athlete's foot, as well as fungal infections of the scalp, fingernails, and toenails. However, its bioavailability is limited by poor water solubility. This work was aimed at improving the aqueous solubility of the drug *via* Polysorbate 80 - modulated nanocrystallization. Two types of nanocrystals (GRFV3 and GRFV2) were generated, respectively, with and without the inclusion of Polysorbate 80 in the nanocrystallization process. The three forms of the drug were subjected to a flowability test, differential scanning calorimetry, Fourier transform infrared spectroscopy, particle size analysis, and aqueous solubility determination. The flowability of the particles was in the order GRFV3 > GRFV2 > GRFV1. The thermogram of the drug powder showed a sharp endothermic transition with a peak at 75 ºC; that of the nanocrystals GRFV2 showed a single sharp endothermic transition with a peak at 170 ºC, while that of GRFV3 showed two endotherms with peaks at 75 ºC and 164 ºC. The nanocrystallization with or without a surfactant did not result in any observable change in the chemical integrity of the drug. The mean particle size was in the order GRFV3 < GRFV2 < GRFV1. There was a significant difference ($P < 0.0001$) in the aqueous solubility of the three forms of the drug, with GRFV3 having the highest value. Polysorbate 80 - modulated nanocrystallization can increase the aqueous solubility of griseofulvin by 4.14 folds; it is a potential way of improving the bioavailability of the drug.

Keywords: Polysorbate 80; nanocrystallization; griseofulvin; aqueous solubility

Introduction

Griseofulvin is a mycotoxin drug that is active against many dermatophytic fungi of different species in the genera Microsporum, Trichophyton, and Epidermophyton [1]. Hence, it is used in the treatment of many infections, including ringworm and athlete's foot, as well as fungal infections of the scalp, fingernails, and toenails. Researchers have observed that among antifungal medications for tinea capitis, griseofulvin, and terbinafine had the best clinical outcomes [1]. Griseofulvin is also indicated in onychomycosis; however, it has largely been replaced with newer agents such as terbinafine, itraconazole, and fluconazole [2].

Due to its capacity to concentrate in the keratinous layer of the epidermis and its relatively low toxicity in men, griseofulvin has been extensively used by oral administration in the therapy of many fungal infections [3]. It is, however, poorly water-soluble, and the absorption from the gastrointestinal tract in humans is slow, erratic, and incomplete [4]. The oral bioavailability is 50-80%, while the peak plasma level occurs 3-4 h after oral administration [5,6].

The clinical failure of the conventional oral therapy of griseofulvin has been attributed to its poor solubility and variable delivery from different commercial products [6]. According to Shegokar and Muller [7], the poor aqueous solubility of some existing drugs, the low oral bioavailability of many new drugs, and general delivery problems are becoming serious challenges. Griseofulvin is poorly water-

soluble and has been categorized as a BCS (Biopharmaceutics Classification System) Class II drug [4]. It has a low solubility but a high permeability; therefore, the bioavailability is only dissolution rate-limited. Even though the drug is relatively old, addressing its characteristic low aqueous solubility may bring a new dawn to this antifungal agent. Various methods are available for improving the solubility of drugs. Two of such methods are size reduction and inclusion of surfactants [8,9]. Besides, the two processes are known to improve drug absorption [10]. The efficiency of absorption from an ultra-microsize form of griseofulvin is approximately double that of the conventional microsize form [11].

Previous work on dissolution assessment of canonized hydrochlorothiazide and nanoparticles developed by precipitation of the drug in the presence of Polysorbate 80 showed that the two substances are not significantly different and that both are better than the unprocessed drug in terms of solubility [12]. Polysorbate 80 is a non-ionic surfactant, and its maximum allowable daily intake is 25 mg/kg [13]. This present work is aimed at improving the solubility of griseofulvin by Polysorbate 80 - modulated nanocrystallization for the purpose of potentially optimizing the delivery of the drug. The drug will be precipitated from the solution; hence, only a small amount of the polysorbate can go with it.

Materials and Methods

Materials

The materials used for this work include griseofulvin powder (Thosco, Thode and Scobel, Hamburg, Germany), dimethyl formamide (Guangdong Guanghua Sci-Tech Co. Ltd., China), and polysorbate 80 (BDH Chemicals, England).

Methods

Preparation of griseofulvin nanocrystals

Griseofulvin nanocrystallization with and without the inclusion of a surfactant was carried out using the antisolvent precipitation technique. Dimethyl formamide was used as the solvent, while water was used as the antisolvent. For the preparation of nanocrystals without the use of a surfactant, 10 g griseofulvin powder was weighed and transferred into a 250 ml capacity beaker. Dimethyl formamide (100 ml) was added to the griseofulvin inside the beaker. The beaker was placed on a magnetic stirrer, and homogenization of the drug and the solvent was done at 500 rpm for 45 min. The homogenized system was transferred into a bigger container, followed by the addition of 500 ml of water for precipitation. The entire content was transferred into a separating flask, and the system was allowed to stand for 1 h for proper separation. The precipitate was gently separated from the supernatant, air-dried for 3 h, and then dried in an oven at 60 ºC for 8 h to form griseofulvin crystals [12]. The preparation was repeated one more time so as to derive a sufficient quantity of the crystals. Nanocrystallization with the inclusion of Polysorbate 80 was also carried out using the same method described above; the only difference in the procedure was the addition of 5 gm Polysorbate 80 to the 10 gm griseofulvin before the solvent (100 ml dimethyl formamide) was added. The procedures of homogenization, precipitation, and drying were carried out as with nanocrystallization without the inclusion of a surfactant. The preparation was repeated one more time so as to derive a sufficient quantity of nanocrystals.

Calculation of percentage yield

The weight of the nanocrystals was taken, and the percentage yield was calculated relative to the initial weight of the griseofulvin powder used for the nanocrystallization.

Determination of flow properties

The conventional method, as described in the work of Olorunsola and Usungurua [14], was used to determine the flow properties of griseofulvin powder and the two types of nanocrystals. A 10 gM sample was placed in a 25 mL measuring cylinder, and the bulk volume was taken. The system was tapped 100 times, after which the volume was retaken. The bulk density (BD) and tapped density (TD) were calculated as the ratio of the mass of the sample to the corresponding volume.

The Carr's index (CI) and Hausner's ratio (HR) were calculated using the two equations below:

$$CI = \frac{TD - BD}{TD} \; X \; 100\ \% \qquad (1)$$

$$HR = \frac{TD}{BD} \qquad (2)$$

Differential scanning calorimetry

The thermal behavior of the powder and the two types of crystals was studied by differential scanning calorimetry using the method described by Olorunsola *et al.* [15]. The thermogram of each sample placed in an A1 40 µL crucible was obtained using a DSC-204 F1 machine (NETZSCH Co., Germany). The scanning was done at 20 ºC per minute heating rate over a temperature range of 35-300 ºC.

Fourier transform infrared spectroscopy

An adequate quantity of sample was placed in a potassium bromide disk, and the spectrum was recorded over a scanning range of 500 to 4,000 cm^{-1} using a spectrophotometer (model 8400S, Shimadzu Corporation, Kyoto-Japan) [15]. The spectra of the drug powder and the two types of nanocrystals were taken using this method.

Particle size analysis and polydispersity index determination

Particle size distribution and polydispersity index (PDI) of the samples were studied using a Zetasizer Nano-ZS (Malvern Instruments, UK). A suitable quantity of each sample was diluted and transferred into the cuvette, and the diluted sample was studied by means of dynamic light scattering using the Zetasizer.

Determination of aqueous solubility

A 0.01 gm quantity of griseofulvin was weighed and transferred into a beaker. Dimethyl formamide (100 ml) was measured and transferred into the beaker for dissolution of the drug. The solution was stirred vigorously, producing a 100 µg/ml stock solution. From the stock solution, 25, 20, 15, 10, and 5 µg/ml solutions were prepared. The absorbances of these solutions taken at 291 nm were used to obtain the Beer-Lambert plot of the drug [16]. A 0.1 gm quantity of griseofulvin powder was weighed and transferred into a measuring cylinder containing 10 ml of purified water. The cylinder was shaken vigorously and then allowed to stand for 30 min, after which the solution was filtered. The same procedure was repeated for the griseofulvin nanocrystals generated without the inclusion of a surfactant and also for the nanocrystals generated with the inclusion of a surfactant. The absorbance of each filtrate was taken at 291 nm, and the value was extrapolated to obtain the solubility of the sample.

Table 1. Parameters relating to the flow properties of griseofulvin powder and the nanocrystals.

S/N	Drug type	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's index (%)	Hausner's ratio
1	GRFV1	0.48 ±0.02	0.75 ± 0.02	35.84 ± 1.88	1.51 ± 0.03
2	GRFV2	0.47 ± 0.01	0.66 ± 0.02	29.18 ± 3.04	1.41 ± 0.06
3	GRFV3	0.36 ± 0.00	0.50 ± 0.02	27.24 ± 3.08	1.38 ± 0.06

GRFV1 = Drug powder, GRFV2 = Nanocrystals generated without the inclusion of a surfactant, GRFV3 = Nanocrystals generated with the inclusion of a surfactant

Results and Discussion

A yield of 73.44% griseofulvin nanocrystals was obtained from nanocrystallization without the inclusion of a surfactant, while 88.72% was obtained from nanocrystallization with the inclusion of Polysorbate 80. The inclusion of the surfactant enhanced the yield of nanocrystals from the griseofulvin powder. Surfactants can increase the solubility of a substance and also enhance the stability of dispersed systems [17]. Since a higher yield was obtained with the inclusion of the surfactant, it can be inferred that Polysorbate 80 facilitated the initial solubilization of griseofulvin in dimethyl formamide so that much of the nanocrystals could be precipitated on the addition of water (the antisolvent used).

Flow properties

The parameters relating to the flow properties of the drug powder and the two types of nanocrystals are shown in Table 1. The bulk density and tapped density were in the order GRFV1 > GRFV2 > GRFV3. It can, therefore, be inferred that the nanocrystallization led to a decrease in densities, and the reduction was further enhanced when the surfactant was introduced during the process of nanocrystallization. Similarly, Carr's index and Hausner's ratio were in the order of GRFV1 > GRFV2 > GRFV3. Both parameters are indirect measures of flowability [18]. Hence, the process of nanocrystallization improved the flow property of griseofulvin. The inclusion of the surfactant led to further improvement in the flow property. Since flowability is important in drug formulation, especially tableting, nanocrystallization with the inclusion of a surfactant improves the processability of griseofulvin.

Differential scanning thermograms

The differential scanning thermograms of the drug powder, nanocrystals generated without a surfactant, and nanocrystals generated with the inclusion of a surfactant are shown in Figure 1, with the exothermic direction being upward. The thermogram of the drug powder showed a sharp endothermic transition with a peak at 75 ºC. The thermogram of the nanocrystals generated without the inclusion of a surfactant also showed a single sharp endothermic transition but with a peak at 170 ºC. The single endothermic transition in both thermograms can be linked to enthalpic relaxation [15]. From the values of the endothermic peaks in both thermograms, nanocrystallization led to an increase in the enthalpic relaxation temperature. On the other hand, the thermogram of GRFV3 (nanocrystals generated with the inclusion of a surfactant) showed two endotherms with peaks at 75 ºC and 164 ºC. The first endotherm corresponds to that of the endotherm of the unprocessed griseofulvin, while the second one corresponds to that of GRFV 2. It can thus be inferred that nanocrystals generated with the inclusion of the surfactant may not be as refined as those generated without the inclusion of the surfactant.

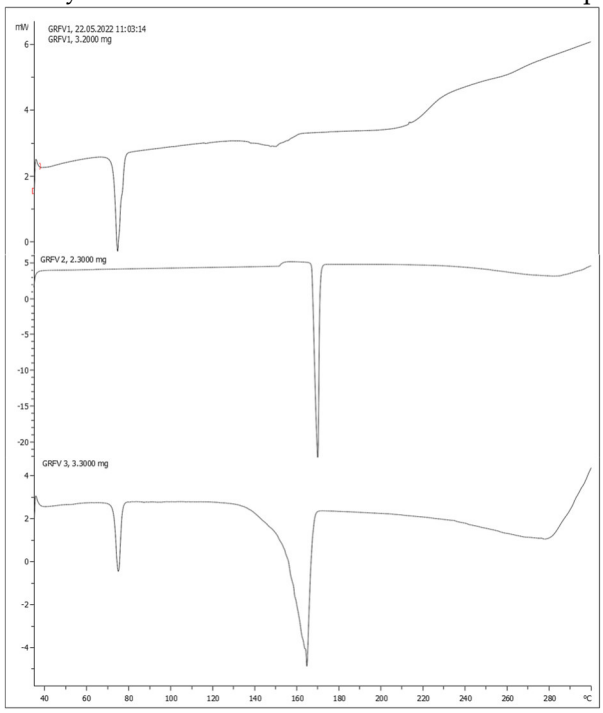

Figure 1. DSC thermograms of GRFV1, GRFV2 and GRFV3 (GRFV1 = griseofulvin powder; GRFV2 = griseofulvin nanocrystals prepared without the inclusion of a surfactant; GRFV3 = griseofulvin nanocrystals prepared with inclusion of a surfactant)

Fourier transform infrared spectra

The Fourier transform infrared spectra of the unprocessed drug powder, nanocrystals generated without a surfactant, and nanocrystals generated with the inclusion of a surfactant are shown in Figure 2. The absorption peaks observed in the three spectra are essentially the same in terms of position and intensity. Hence, the nanocrystallization with or without a surfactant did not lead to observable changes in the chemical integrity of the drug.

Particle size distribution and polydispersity index

Particle size distribution and polydispersity index of the pure powder and those of the two types of nanocrystals are shown in Figures 3 a-c. The Z-average (average diameter of the particles) was in the order GRFV3 < GRFV2 < GRFV1 where GRFV1 = griseofulvin powder; GRFV2 = griseofulvin nanocrystals generated without the use of a surfactant; GRFV3 = griseofulvin nanocrystals generated with the inclusion of Polysorbate 80.

There was no significant difference in the polydispersity index of the powder and those of the two forms of nanocrystals. However, there was a reduction in the particle size with nanocrystallization. The nanocrystallization process brought forth a 22.4% reduction in particle size (Z-average of 100.2 to 77.8 nm).

With the inclusion of Polysorbate 80 during nanocrystallization, the process brought forth a 55.5% particle size reduction (from a Z-average of 100.2 to 44.7 nm). This implies that the inclusion of the surfactant produced a significant difference, causing a further reduction from 77.8 to 44.7 nm, which is equivalent to a 33.1% further reduction in the particle size of the drug. Therefore, the inclusion of a surfactant is very effective in enhancing the nanocrystallization of griseofulvin. The inclusion of the surfactant not only caused an increase in the yield of the nanocrystals but also modulated the particle size distribution, promoting the formation of crystals of small sizes. This observation is in agreement with the report of Muller *et al*. [19].

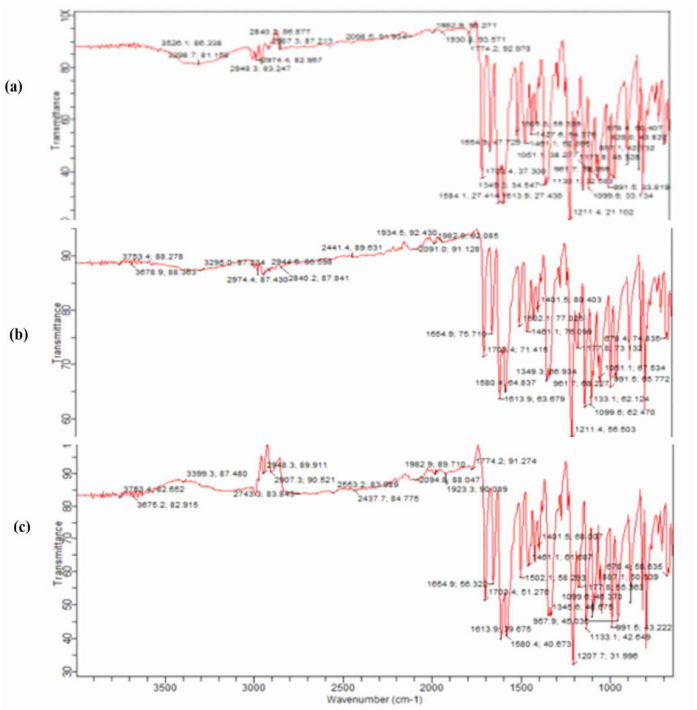

Figure 2. FTIR spectra of (a) Unprocessed griseofulvin powder; (b) Griseofulvin nanocrystals prepared without a surfactant; (c) Griseofulvin nanocrystals prepared with a surfactant.

Aqueous solubility

The aqueous solubility of the pure drug powder and those of the two types of nanocrystals are shown in Table 2.

Table 2. Aqueous solubility of griseofulvin powder and the nanocrystals.

S/N	Drug type	Aqueous solubility (mg/l)	Fold increase in solubility relative to the drug powder
1	GRFV1	2.96 ±0.01	-
2	GRFV2	5.09 ± 0.01	1.72-fold
3	GRFV3	12.25 ± 0.01	4.14-fold

GRFV1 = Drug powder, GRFV2 = Nanocrystals generated without the inclusion of a surfactant, GRFV3 = Nanocrystals generated with the inclusion of a surfactant

There was a significant difference ($P < 0.0001$) in the aqueous solubility of the three forms of the drug, with GRFV3 (the crystals generated with the inclusion of Polysorbate 80) having the highest aqueous solubility. Nanocrystallization without the inclusion of the surfactant brought forth a 1.72-fold increase in aqueous solubility, while nanocrystallization with the inclusion of the surfactant brought forth a 4.14-fold increase in the aqueous solubility. Hence, the effect of the inclusion of the surfactant is more than double that of nanocrystallization alone.

Particle size reduction is one of the strategies and approaches that can be used to improve the solubility of poorly water-soluble drugs such as griseofulvin [20-22]. Also, earlier works have shown that griseofulvin is one of the drugs whose absorption is highly improved by particle size reduction [23]. Therefore, nanocrystallization with the inclusion of Polysorbate 80, having the ability to increase the aqueous solubility of griseofulvin by 4.14 folds, is a potential way of improving the bioavailability of the drug. Further work should be carried out to assess the drug delivery from tablet formulations containing the nanocrystals.

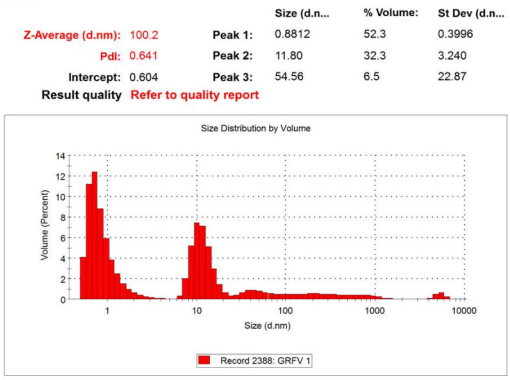

Figure 3a. Particle size distribution and polydispersity index of griseofulvin powder.

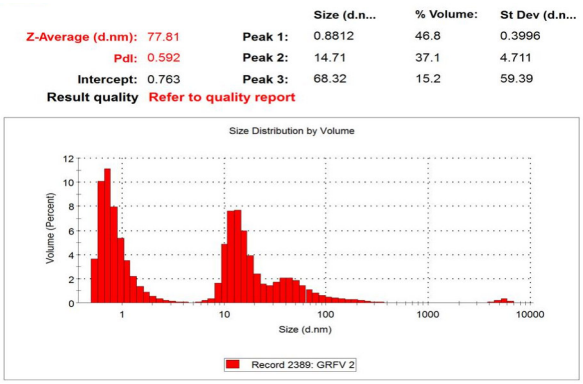

Figure 3b. Particle size distribution and polydispersity index of griseofulvin nanocrystals generated without the inclusion of a surfactant.

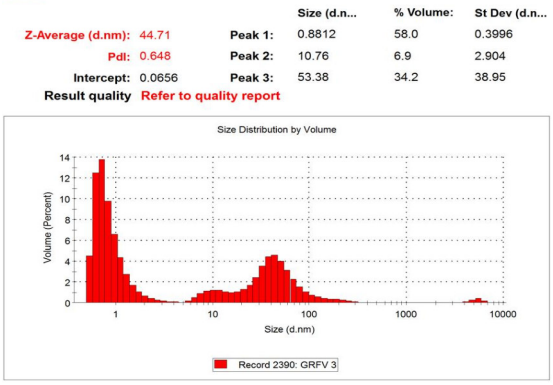

Figure 3c. Particle size distribution and polydispersity index of griseofulvin nanocrystals generated with the inclusion of Polysorbate 80.

Conclusion

Polysorbate 80 - modulated nanocrystallization produces a higher yield of griseofulvin nanocrystals compared to ordinary nanocrystallization. It also causes better improvement of the flow property of the drug. Furthermore, nanocrystallization with or without a surfactant does not result in a significant change in the chemical nature of griseofulvin. The mean particle size is in the order GRFV3 < GRFV2 < GRFV1. Nanocrystallization without the inclusion of Polysorbate 80 produces a 1.72-fold increase in aqueous solubility, while nanocrystallization with the inclusion of the surfactant produces a 4.14-fold increase in aqueous solubility. Polysorbate 80 - modulated nanocrystallization increases the aqueous solubility of griseofulvin significantly. It is a potential way of improving the delivery and bioavailability of the drug.

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All the authors gave final approval of the version of the manuscript to be published. There is agreement among the authors to be accountable for all the aspects of the work.

Authors contribution

All the authors have contributed equally.

Declaration of interest

The authors declare no conflict of interest.

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