Comparing dissolution, solubility and trans-membrane flux of nanoparticle formulation of griseofulvin with micronized and un-processed drug

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INTRODUCTION

Recently developed Zero Intercept Method[1] (ZIM) enabling, for example, *in situ* concentration monitoring of free API being released from nanoparticles was applied to determine solubility and dissolution of gresiofulvin formulated as nanosuspension. The effect of formulation was also studied through miniaturized dissolution-permeability setup (µFLUX) to determine if nanosuspension formulation improves flux of the griseofulvin through artificial membrane.

MATERIALS AND METHODS

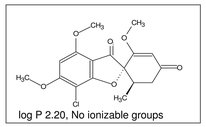

Figure 1. Chemical structure of GSF

Untreated powder of griseofulvin (GSF-Untreated) was purchased from Sigma (St. Louis MO, USA); see chemical structure in Figure 1. GSF-Micronized was prepared by jet-milling technology using powder of GSF purchased from Chifeng Pharma (Raleigh NC, USA). GSF-Microsuspension was prepared by suspending GSF-Micronized (10% w/w) in a mixture of HPMC (2.5% w/w), SLS (0.5% w/w) and deionized water. GSF-Nanosuspension was prepared by wet-media milling technology using suspended GSF-Micronized (10% w/w) in a mixture of HPMC (2.5% w/w), SLS (0.2% w/w) and deionized water.

The particle size of the GSF-Micronized was characterized using laser light diffraction. For the nanosuspension the dynamic light scattering was used for particle size determination. Morphology of the samples was studied using scanning electron microscopy (SEM).

The flux of different forms of GSF through artificial membranes was studied *in situ* using the µDISS Profiler™ ((Pion, Billerica MA, USA, Figure 2) equipped with µFLUX apparatus (Pion, Figure 3), an add-on module consisting of four pairs of temperature controlled side-by-side permeability chambers mounted on top of the stirring platform.

Figure 2. The µDISS Profiler monitors concentration in real time with ability to to dynamically change media in 8 temperature controlled vessels using only 1 – 20 mL.

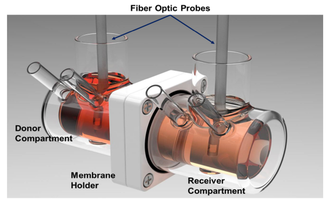

Figure 3. A schematic of the µFLUX apparatus showing a pair of the donor and receiver chambers. FO probes attached to the µDISS Profiler monitor concentrations in the donor (left) and receiver (right) compartments. The chambers can be separated by artificial, cell-based size exclusion, or other types of membranes mounted in the Membrane Holder.

Each pair (Figure 3) consists of a donor and an receiver compartment separated by a filter-supported membrane (e.g., Caco-2, MDCK, PAMPA, dialysis, etc.) with 1.5 cm² area. In this study GIT-optimized artificial membrane (Double-Sink™ PAMPA[2]) was used. The donor compartment is filled with 16 mL of the media of interest (DI water) while the receiver compartment contained Acceptor Sink Buffer at pH 7.4 (ASB-7.4, Pion Inc). The integrated fiber-optic UV probes were positioned in the donor and receiver compartments allowing real time concentration monitoring in all chambers.

Zero Intercept Method (ZIM) analysis was performed using Au PRO™ software version 5.1 (Pion).

Particle Size, Morphology and Crystallinity

Table 1. Particle size distribution in GSF-Micronized.

Griseofulvin	x_{10} µm	x_{50} µm	x_{90} µm
GSF-Untreated	?	?	?
GSF-Micronized	1.0	4.2	13.9

Table 1 summarizes particle sizes (volume weighted) for the micronized powder of GSF. Particle size for the GSF-Untreated was not determined during this study. The mean particle size in the nanosuspension was determined to be 98 nm with a polydispersity index of 0.2. Morphology of the powders and suspensions is presented in Figure 4.

XRPD analysis confirmed the same crystalline structure for micronized and nano-sized GSF, see Figure 5. Slight shift of the peaks is related to the processing of the material while peak broadening reflects the nano-feature of the material.

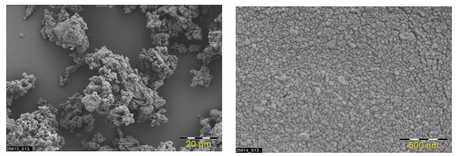

Figure 4. SEM images of GSF-Micronized (left) and GSF-Nano-sized (right).

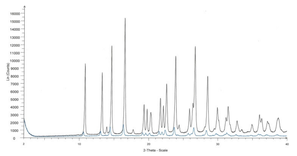

Figure 5. X-Ray characterization of GSF-Micronized (black pattern) and GSF-Nanosuspension (blue pattern).

RESULTS AND DISCUSSION

Zero Intercept Method (ZIM™)

When nanoparticles are present in the solution they absorb light[1,3] effectively acting as another component in addition to the dissolved API. To determine concentration of dissolved API in the presence of nanoparticles a special analysis of the second derivative spectrum (*Zero Intercept Method*, ZIM™) was developed[1,4]. In ZIM, the standard curve for nanoparticles is built by plotting 2nd derivative absorbance values at wavelengths where values of 2nd derivative spectra of fully dissolved API, $AU''_{API}(\lambda_{ZIM})$ equal to 0 (intercepts wavelength axis, Figure 6) versus amount of nanoparticles added. At these special wavelengths only nanoparticles contribute to the second derivative spectrum, i.e.: $AU''_{Total}(\lambda_{ZIM}) = AU''_{Nano}(\lambda_{ZIM})$. The plot of $\sum|AU''_{Total}(\lambda_{ZIM})|$ versus load of API from nanosuspension (µg/mL) will consist of horizontal portion where all nanoparticles dissolve and a sloping line when both dissolved API and nanoparticles are present in the media, but only nanoparticles contribute to the derivative spectra, see Figure 7 for GSF-Nanosuspension.

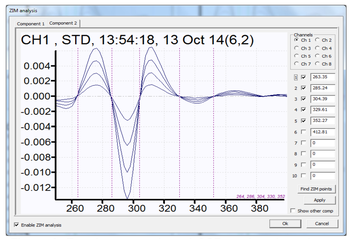

Figure 6. Zero intercept points of GSF used to determine solubility of GSF-nano-sized.

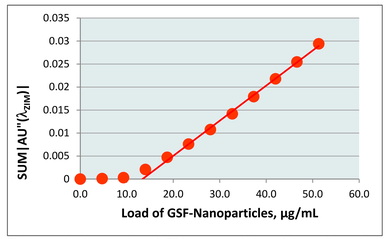

Figure 7. Implementation of ZIM – extrapolation of linear fit to the concentration axis indicates solubility of GSF-nano-sized.

Intercept of the fitted line with concentration axis will give solubility of the nanoparticles in the solution. Figure 8 shows solubility of GSF from nanosuspension determined *in situ* by ZIM for 8 measurement channels and comparison with measurement done by equilibrium dialysis.

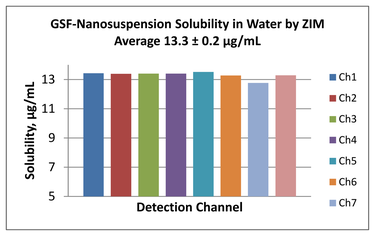

Figure 8. Solubility of GSF nanosuspension in DI water for 8 channels (replicates).

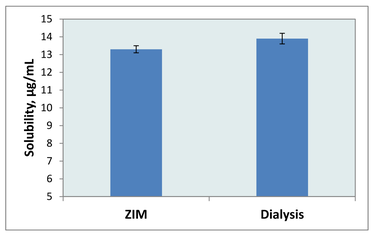

Figure 9. Comparing solubility of GSF nanosuspension in DI water determined *in situ* by ZIM and using equilibrium dialysis method.

Solubility of GSF-Nanosuspension determined by ZIM agreed well with the value obtained from equilibrium dialysis experiment using 10 – 12 KDa cut-off filters.

Simultaneous In Situ Dissolution and Permeation Monitoring

Effect of micronization on potential absorption was studied using µFLUX apparatus.

Figure 9 shows example of the dissolution and appearance profiles for various forms of GSF.

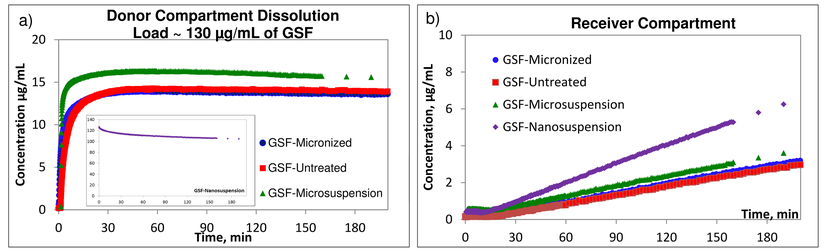

Figure 9. Dissolution (a) and appearance (b) profiles (µg/mL versus min) for various forms of GSF. Insert in the Figure 9 a) shows decrease in the amount of nanoparticles due to dissolution and permeation.

Flux (µg/(min·cm²) of the substance through membrane with area A (cm²) into the vessel with volume V (mL) can be determined by using the following equation:

$$Flux = \frac{dm}{A \cdot dt} = \frac{V}{A} \cdot \frac{dc}{dt} \qquad (1)$$

The slope of profiles, i.e. *dc/dt* shown in Figure 9 b) can be used to measure the flux of GSF through artificial membrane.

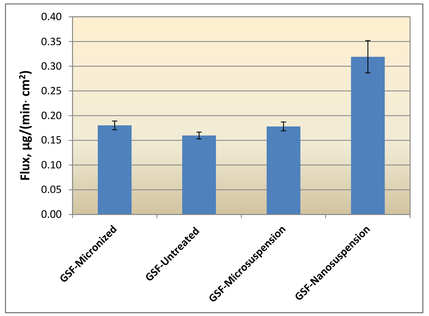

Figure 10 visualize the flux values for different forms of GSF. It is evident that micronization of GSF did not lead into any significant increase in the flux of the GSF through artificial membrane while flux from nanosuspension was significantly higher than from any other studied forms of GSF.

Figure 10. Flux values measured by fitting concentration – time profiles in the receiver compartment (30 – 120 min) with straight line and using equation (1) to convert the slopes of the lines into Flux .

CONCLUSIONS

The study confirmed ability of the ZIM to determine solubility of the nanoparticles *in situ* with no need for solid separation.

Solubility of GSF from nanosuspension was the same as from powdered forms.

Dissolution-permeability study of different forms of GSF suggested that micronization of the GSF powder will not affect the absorption potential of the drug while creating nanosuspension of GSF may lead to improvement of its pharmaco-kinetic properties.

µFLUX apparatus expends potentials of *in situ* concentration monitoring by providing invaluable insight into effect of formulations on all three key physicochemical parameters: dissolution, solubility and flux of the material through membranes.

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