MICRONISATION OF GRISEOFULVIN BY THE AEROSOL SOLVENT EXTRACTION SYSTEM

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ABSTRACT

In this study, griseofulvin was micronised by the Aerosol Solvent Extraction System in order to improve the dissolution profile and enable more safe and effective delivery. The effects of process variables, such as temperature, pressure, solute concentration and solvent type, on griseofulvin precipitation were evaluated.

Micronisation of griseofulvin by the ASES process produced needles that ranged in size from 10 μm to many millimeters. Griseofulvin was precipitated from acetone, ethanol and dimethylformamide, with the highest yield attained in the ethanol system. Ethanol is more suitable for pharmaceutical applications, compared to solvents that have been previously used to micronise griseofulvin.

Preliminary studies indicate that there is significant improvement in the dissolution rate of griseofulvin in water when micronised by the ASES proces. The significance of these results is that the efficacy of the griseofulvin dosage forms may now be improved.

INTRODUCTION

Griseofulvin is an oral antibiotic used in the treatment of fungal infections, the administration of which is limited due to the poor water solubility and low bioavailability of the drug. The poor absorption of the drug in the gastrointestinal tract means that a large amount of a dose of griseofulvin is excreted unchanged.[1] Therefore, in this study, griseofulvin was micronised in order to improve the dissolution profile and increase the bioavailability. The processing of griseofulvin will enable more effective and safe delivery of the drug, since the required dosage amount may be decreased and side effects reduced.

Conventional techniques for micronisation are unsuitable for use with many pharmaceuticals since the thermal and mechanical stresses can degrade the drug. These techniques can also be time consuming and produce particles with a wide particle size distribution and unacceptable levels of residual solvent. Therefore, in this study, the feasibility of processing griseofulvin by the Aerosol Solvent Extraction System (ASES) was investigated.

In the ASES process, carbon dioxide acts as a dense gas anti-solvent to precipitate a solute from a liquid solvent. A dense gas is a material close to or above the critical point, generally with a reduced temperature and pressure between 0.9 and 1.2. Dense gases have unique properties that can be used to produce micronised particles with a narrow particle size distribution and minimal residual solvent at moderate temperatures.

The solubility of griseofulvin in carbon dioxide has been previously studied and was found to be 1.6×10^{-5} mole fraction at 50°C and 200 bar.[2] It is therefore evident that the very low solubility of griseofulvin makes it a suitable candidate for anti-solvent techniques using supercritical carbon dioxide, such as the ASES process. Reverchon and Della Porta previously

studied the micronisation of griseofulvin by Supercritical Anti-Solvent (SAS) precipitation, which follows the same method as the ASES process.[3] While precipitation of griseofulvin from *N*-methyl-2-pyrrolidone (NMP) solutions was unsuccessful, due to almost complete extraction of the drug, long needle-like crystals were produced when dichloromethane (DCM) and dimethyl sulfoxide (DMSO) were used as solvents.[3] Therefore the nature of the solvent has a significant effect on the precipitation of griseofulvin in the ASES technique.

Chattopadhyay and Gupta have used a modified ASES process, Supercritical Anti-Solvent precipitation with Enhanced Mass transfer (SAS-EM), to process griseofulvin.[4] The SAS-EM process incorporates a surface vibrating at ultrasonic frequencies to atomise the jet into small micro droplets in order to improve mass transfer, increase mixing and decrease agglomeration. It was found that variation of the vibration intensity could alter the size and morphology of griseofulvin micronised from dichloromethane (DCM) and tetrahydrofuran (THF) solutions. The morphology changed from long needles, when no ultrasound was used, to nearly spherical nanoparticles, as small as 130 nm, when high vibration intensity was utilised.

Dense gas processes that require the solute to be soluble in the supercritical fluid, such as Rapid Expansion of Supercritical Solutions (RESS), have also been used to process griseofulvin, using trifluoromethane as the supercritical solvent.[2, 5] Reverchon *et al.* found that processing of griseofulvin by the RESS process produced long needles, 13 – 37 μm in length and 1 – 1.3 μm diameter, and quasi-spherical particles with diameters ranging from 0.9 – 1.4 μm.[2] Turk *et al.* produced griseofulvin particles, with a diameter between 200 – 300 nm, which showed significant improvement in the dissolution rate and hence bioavailability of griseofulvin processed by the RESS technique.[5] The RESS process is advantageous for pharmaceutical processing, since there is no organic solvent requirement. However, for processing griseofulvin, extreme conditions between 60 – 150°C and 180 – 300 bar were required. Therefore, the ASES technique is the preferred processing step for griseofulvin.

MATERIALS AND METHODS

Materials: Lab grade griseofulvin was obtained from Sigma Chemical Company. Acetone (Chem Supply, lab reagent grade, 99% purity), ethanol (Chem Supply, 99% purity), N,N-dimethylformamide (Ajax Chemicals, HPLC grade) and carbon dioxide (Linde Gas Pty. Ltd.) were used in the precipitation experiments.

Method: The ASES set-up used in this study was the same as that described by Warwick *et al.*[6] In all experiments, carbon dioxide was used as the dense gas anti-solvent. Griseofulvin was precipitated from dimethylformamide (DMF), ethanol and acetone solutions, at temperatures between 10 and 40°C, and pressures between 52 and 190 bar. Solute concentration was varied between 0.7 and 10 wt %. A solvent flowrate of 0.1 - 0.2 mL/min., minimum solvent/anti-solvent flowrate ratio of 1:25 (anti-solvent flowrate varying from 5 – 21 mL/min.) and nozzle diameter of 50 - 250 μm was used. The effects of process variables, such as temperature, pressure or carbon dioxide density, solute concentration and solvent type, on the particle size and morphology of precipitated griseofulvin were evaluated.

The particle size and morphology of the precipitated particles was examined using a Scanning Electron Microscope (SEM, Hitachi S-4500II Field Emission SEM). The samples were prepared for the SEM by vacuum coating with 1 – 2 nm chromium using the Xenosput.

The effect of micronisation on the dissolution profile of griseofulvin was investigated by powder dissolution using the USP paddle method and the Vankel 7000 Dissolution Equipment, with a Cary UV 50 Spectrophotometer. The dissolution media was 900 – 1000 mLs of deionised water, at 37°C, with 700 mg of surfactant (sodium dodecyl sulfate) added to

aid the dissolution process. A stirring rate of 50 rpm was used. Samples were withdrawn at regular time intervals and the UV absorbance was measured at 295 nm.

RESULTS AND DISCUSSION

Micronisation Study: Unprocessed griseofulvin consists of irregular particles ranging in size from 1 – 20 μm, as shown in Figure 1a. Processing of griseofulvin by the ASES technique produced needles and some large crystals at all conditions investigated, as shown in Figure 1b. The needles ranged in size from 10 μm to many millimetres. The surface area has been significantly changed by the production of long, thin needles and this may have a dramatic effect on the dissolution profile.

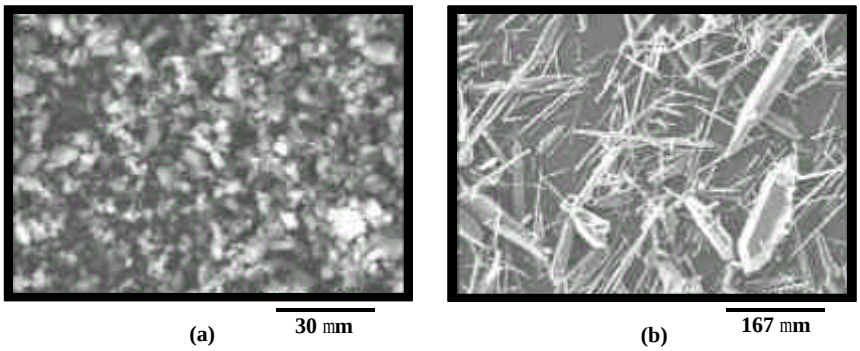

Figure 1: (a) Unprocessed griseofulvin, **(b)** Griseofulvin micronised by ASES at 25°C and 142 bar, with a solute concentration of 3.3 wt % in acetone and a 250 μm nozzle.

The type of solvent significantly impacted the yield of the ASES process. The yield of griseofulvin precipitate was extremely low when DMF was used as a solvent. Since the solubility of griseofulvin in carbon dioxide is limited, the presence of DMF may have increased the solubility and the mxiture may be in a homogeneous region of the ternary phase diagram of griseofulvin-CO_2-DMF. The yield was improved for ethanol and acetone solutions, with the highest yield being obtained for the ethanol system.

Reverchon and Della Porta have made similar observations when precipitating griseofulvin from DCM, DMSO and NMP.[3] It was observed that very long needles, up to a few millimeters in length, were produced from DCM and DMSO solutions. However, when NMP was used, griseofulvin was almost completely extracted and only traces were found in the precipitation chamber. Reverchon and Della Porta attributed the extraction of griseofulvin to the formation of a solvato-complex and the increased solubility of the drug in the anti-solvent, commonly referred to as the co-solvent effect. It can be seen that a similar product, consisting of long needles, was formed in this study. The added benefit is that less toxic solvents have been used, compared to the work of previous authors.

The effect of temperature on the precipitation of griseofulvin from acetone solutions was investigated. The temperature was varied between 10 and 40°C, while the carbon dioxide density was kept constant. Increasing the temperature increased the size of the needles produced and improved the yield of the process. The shorter needles produced at 10°C are shown in Figure 2a. The effect of changing carbon dioxide density was then investigated for the acetone system, while maintaining all other parameters constant. At 40°C, an increase in pressure from 90 to 190 bar improved the yield of the process and produced longer and more uniform needles, as shown in Figure 2b. An increase in griseofulvin concentration in acetone also improved the yield of the process. At very low concentrations, such as 1.8 wt % in acetone

solution, no precipitation was observed. The change in concentration had a negligible effect on the size and morphology of the micronised griseofulvin.

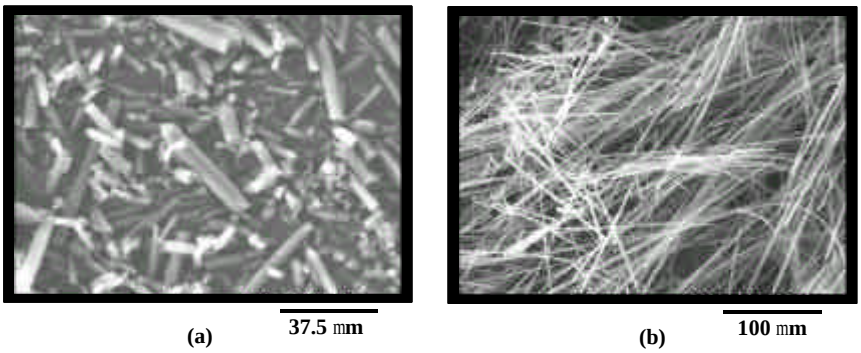

Figure 2: Griseofulvin micronised from acetone by ASES using a 250 μm nozzle at: **(a)** 10°C and 53 bar, **(b)** 40°C and 190 bar.

Dissolution Study: Comparison of the dissolution rate of unprocessed griseofulvin and ASES micronised griseofulvin was conducted. The dissolution rate constant, which is equal to the inverse of the time taken for 63.2% of the sample to dissolve, was calculated for the unprocessed and processed griseofulvin and was equal to 0.03 and 0.12 sec^{-1}, respectively. More significantly, 20% more of the processed drug had dissolved in the first 20 mins. compared to the unprocessed sample. Therefore, these results suggest that the re-processing of griseofulvin by the ASES technique has improved the dissolution characteristics of the drug.

The effect of micronisation on the dissolution profile has also been investigated by Turk *et al.* for griseofulvin processed by the RESS technique.[5] The RESS processed griseofulvin showed an improved dissolution rate, in comparison to unprocessed griseofulvin and griseofulvin micronised by a conventional technique. In the first 20 mins. of the dissolution study, approximately 20% more of the processed drug had dissolved in comparison to the unprocessed drug. It is therefore evident that the dissolution rate enhancement is similar for ASES and RESS processing.

CONCLUSIONS

Micronisation of griseofulvin by the ASES technique produced needles, which ranged in size from 10 μm to millimeters. The particle size, morphology and particularly the yield of the precipitated particles were influenced by temperature, carbon dioxide density, solute concentration and especially solvent type.

The dissolution rate of griseofulvin has been significantly improved by ASES processing. The significance of the improvement in the dissolution profile is that the bioavailability of the drug may be increased and therefore more safe and efficient delivery may be possible.

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