# Optimizing Small Scale Nanomilling Process Parameters for Genistein

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## Abstract

**Purpose:** This experiment was conducted to assess the main process variables for small scale bead milling of genistein using the Retsch® Mixer Mill

**Methods:** The Retsch® Mixer Mill MM 301 was used with 50-mL zirconia-lined jars. The suspending medium was composed of 0.2% Polysorbate 80/5% Povidone K17. The grinding beads were 0.3 mm yttria stabilized zirconia. A design of experiments approach was used to create a ½ fraction 4-factor, 2-level, center point design. Particle size was measured by laser diffraction (Malvern Mastersizer 2000).

**Results:** All main effects were statistically significant, with frequency the largest and drug load the smallest effects. There were three interactions that were statistically significant, with drug load-cycles the most important factor. The effect of cycles was opposite for low and high drug loads, with higher drug loads resulting in larger particle size for longer cycles. Using the regression equation, it was found that the most efficient reduction in size transpired with a 40% drug load (high), a suspension volume to bead volume ratio of 1.2 (high), frequency of 16 Hz (high), and 2 cycles (low) of milling.

**Conclusions:** A small scale mixer mill can be used to nanomill genistein to a very fine size. The most important factor is frequency in size reduction. It is also critical to consider that a material can be over milled, which in this experiment transpired with higher drug load and longer cycle time. The DOE ½ fraction design and regression equation enabled a prediction of the optimum response (smallest size) for: high drug load (40%), high volume suspension to bead ratio (1.2-fold), high frequency (16 Hz), and short cycle time (2 cycles). This finding is favorable, in that it is generally desired to mill high drug loads and larger volumes of suspension in the shortest time possible.

## Introduction

Genistein is an isoflavone found in a number of plants, exhibiting antioxidant and other biological activities.[1] The water solubility of genistein is reported to be low, ~1 μg/mL.[2] The molecule's lowest pKa is 7.2,[3] which is too high for significant solubility enhancement by pH adjustment, especially by the parenteral route. Previous work in the Pharmaceutical Experiment Station lab determined genistein's solubility in other vehicles such as cosolvents and lipids to be sufficiently low, which led to the development of nanomilled formulation, using a suspending medium composed of 0.2% Polysorbate 80/5% Povidone K17 (w/w). The nanomilled formulation was required in small quantities repeatedly over ~1 year, which prompted an optimization experiment to determine the milling parameters that would produce genistein efficiently in high yield.

## Materials

Genistein lot SI06068001 (DSM Nutritional Products)
Polysorbate 80 lot E35595 (J.T. Baker)
Povidone K17 lot 05700183681 (ISP Corp.)
House deionized water passed through cartridges for organic removal, cation and anion exchangers and 0.2-μm filtered (Barnstead/Thermo Scientific)
YTZ® grinding media, 0.3 mm, lot 52000180030 (Tosoh Corp.)
Polypropylene Spectra/Mesh® 210 μm macroporous filter lot 3231421 (Spectrum Labs, Inc.)

## Methods

A Retsch® Mixer Mill MM 301 was used with 50 mL zirconia-lined jars and 0.3 mm YTZ beads loaded to 75 g. The suspending medium was composed of 0.2% Polysorbate 80/5% Povidone K17, prepared on a w/w basis. The required weights of genistein and suspending vehicle, which varied for each experimental design run, were filled into the milling jar with the beads. The jar was closed with its lid and set in the equipment at the required frequency and cycle time. The suspension was separated from the beads by gravity filtration with a 210 μm macroporous filter. Particle size was measured by laser diffraction, using the Malvern Mastersizer 2000 and Hydro 2000S small volume disperser, containing deionized water. The Mie model was used with refractive index = 1.73 (genistein value) and absorption = 001. Each sample was run in duplicate and the average is reported.

## Experimental Design

The four main variables in the milling process are drug load (DL), suspension volume/bead volume ratio (VB), oscillation frequency (F) and cycles (C), where each cycle is 99 min. These four factors were examined at 2 levels in a ½ fractional factorial statistical design with three repetitions of a center point. The design and statistical analysis were accomplished using Minitab 15 statistical software (Minitab Inc.).

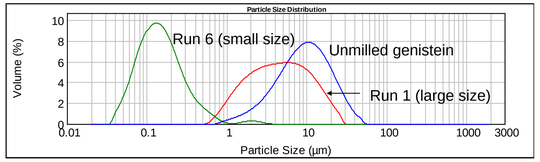
## Results & Discussion

The design and particle size results are summarized in Table 1, with an example (Fig. 1) of the particle size distribution plots for input (unmilled) genistein, a small and large size milled sample.

**Table 1 – DOE with Particle Size Results**

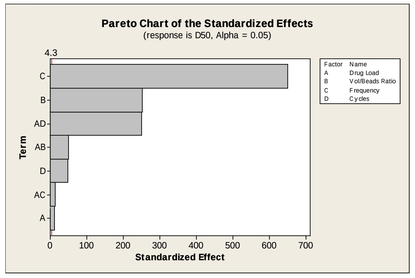
| Run No. | Drug Load (w/w %) | Vol/Beads Ratio | Frequency (Hz) | Cycles | Particle Size (um) D10 | D50 | D90 |
|---|---|---|---|---|---|---|---|
| 1 | 40 | 0.8 | 4 | 6 | 1.251 | 3.591 | 10.508 |
| 2 | 10 | 0.8 | 4 | 2 | 0.131 | 3.315 | 18.422 |
| 3 | 25 | 1 | 10 | 4 | 0.067 | 0.138 | 0.345 |
| 4 | 40 | 1.2 | 16 | 6 | 0.073 | 0.115 | 0.183 |
| 5 | 40 | 0.8 | 16 | 2 | 0.065 | 0.130 | 0.807 |
| 6 | 25 | 1 | 10 | 4 | 0.067 | 0.139 | 0.337 |
| 7 | 40 | 1.2 | 4 | 2 | 0.120 | 1.364 | 7.484 |
| 8 | 25 | 1 | 10 | 4 | 0.066 | 0.130 | 0.279 |
| 9 | 10 | 1.2 | 4 | 6 | 0.103 | 1.827 | 8.276 |
| 10 | 10 | 1.2 | 16 | 2 | 0.070 | 0.118 | 0.200 |
| 11 | 10 | 0.8 | 16 | 6 | 0.077 | 0.117 | 0.187 |

**Figure 1 – Example Particle Size Distribution Results**




The ANOVA results are summarized in the Pareto chart in Figure 2. All effects shown are statistically significant at the 95% confidence level (critical value = 4.3 shown in upper left of plot). The three largest effects are frequency, suspension volume/beads ratio and the drug load-cycles interaction.

**Figure 2 – Pareto Chart for D50 Size Parameter**

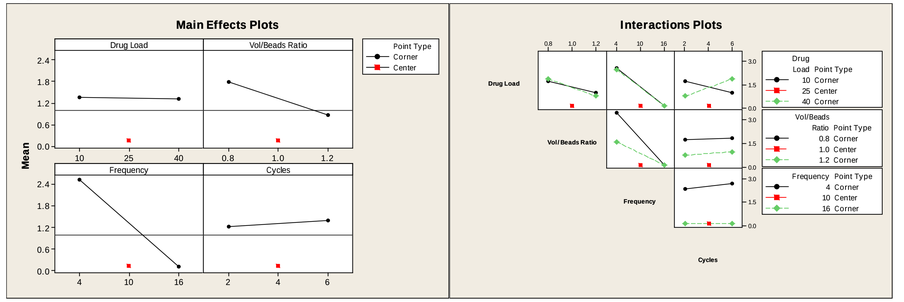



## Results & Discussion (continued)

The experimental design plots for D50 are given in Figure 3, which illustrates the change in particle size broken down into main effects and interactions. The interactions plot shows why the drug load-cycles interaction is important: for low drug load (10%) an increase in cycles reduces size, but at high drug load (40%) an increase in cycles increases size. It is possible to over mill genistein.

The regression equation for the D50 response enables calculation of the factor settings that will produce the smallest size. The regression/optimization calculation predicts that the best size reduction is obtained with: drug load = 40%, suspension volume/beads ratio = 1.2, frequency = 16 Hz, cycles = 2

**Figure 3 – Main Effects and Interactions Plots for D50**




## Conclusions

A small scale mixer mill can be used to nanomill genistein to a very fine size, with D10 = 0.07 μm, D50 = 0.11 μm and D90 = 0.18 μm. The most important factor is frequency in size reduction. It is also critical to consider that a material can be over milled, which in this experiment transpired with higher drug load and longer cycle time. The DOE ½ fraction design and regression equation enabled a prediction of the optimum response (smallest size) for: high drug load (40%), high volume suspension to bead ratio (1.2-fold), high frequency (16 Hz), and short cycle time (2 cycles). This finding is favorable, in that it is generally desired to mill high drug loads and larger volumes of suspension in the shortest time possible.

## References

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