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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: Drug load and frequency were the statistically significant main effects for D50 and only drug load was significant for D90, with lower drug load resulting in smaller particle size. Surprisingly, lower frequency produced smaller particle size. There were important interactions on D50 for drug load-frequency and drug load-cycles. Lower frequency was favorable to smaller D50, but the effect was particularly strong for high drug load. The drug load-cycles interaction was significant in that for low drug loads smaller D50 was obtained at shorter milling time, while the opposite was true for high drug loads. Response optimization found that the greatest reduction in size transpired with 10% drug load (low), suspension to bead volume ratio of 1.5 (high), frequency of 16 Hz (low), and 0.6 cycles (low) of milling. For high drug load, particle size could not be reduced as effectively, but the best operating parameters were identical to low drug load.
Conclusions: In nanomilling genistein, the most important factors are drug load and frequency. It is also critical to consider the important interactions of drug load with frequency and cycles (or time), especially for low drug loads where it is best to use lower frequency and shorter cycles. The optimum response (smallest size) was for low drug load (10%), high suspension to bead volume ratio (1.5-fold), low frequency (16 Hz), and short cycle time (0.6 cycles). For high drug load, particle size could not be reduced as small as for low drug load, although the optimum operating parameters were identical as for low drug load.

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 = 0.001. 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 two repetitions of a center point and of a corner point. The design and statistical analysis were accomplished using Minitab 15 statistical software (Minitab Inc.).

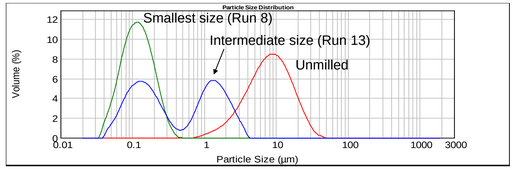
Results & Discussion

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, small and intermediate size milled samples.

Table 1 – DOE with Particle Size Results

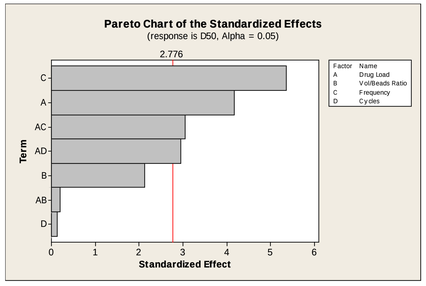
Run No.	Drug Load (w/w %)	Vol/Beads Ratio	Frequency (Hz)	Cycles	Particle Size (um) D10	D50	D90
1	40	1	30	0.6	0.95	0.586	13.317
2	25	1.25	23	1.3	0.083	0.247	5.474
3	10	1	30	2	0.085	0.316	2.149
4	40	1	16	2	0.083	0.183	6.598
5	40	1.5	30	2	0.098	0.405	12.8
6	40	1.5	16	0.6	0.079	0.204	0.937
7	10	1.5	30	0.6	0.064	0.127	0.278
8	10	1	16	0.6	0.062	0.116	0.219
9	25	1.25	23	1.3	0.085	0.28	6.849
10	10	1.5	16	2	0.071	0.155	0.461
11	10	1.25	23	1.3	0.085	0.294	8.327
12	10	1.5	30	2	0.091	0.269	1.681
13	10	1	30	2	0.083	0.319	2.059

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 = 2.776). The four largest effects are drug load, frequency, drug load-frequency and drug load-cycles interactions.

Figure 2 – Pareto Chart for D50 Size Parameter

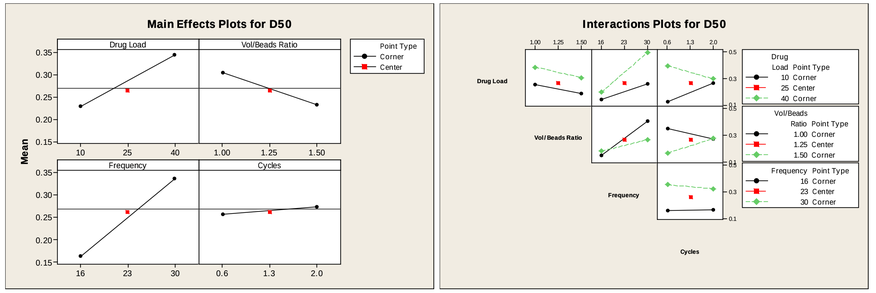


Results & Discussion (continued)

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 high drug load (40%) an increase in cycles reduces size, but at low drug load (10%) 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 = 10%, suspension volume/beads ratio = 1.5, frequency = 16 Hz, cycles = 0.6.

Figure 3 – Main Effects and Interactions Plots for D50



Conclusions

In nanomilling genistein, the most important factors are drug load and frequency. It is also critical to consider the important interactions of drug load with frequency and cycles (or time), especially for low drug loads where it is best to use lower frequency and shorter cycles. Overall, using a frequency as high as 30 Hz is not as effective as 16 Hz. This effect is presumably due to ineffective bead movement at higher frequency. The optimum response (smallest size) was for low drug load (10%), high suspension to bead volume ratio (1.5-fold), low frequency (16 Hz), and short cycle time (0.6 cycles). For high drug load, particle size could not be reduced as small as for low drug load, although the optimum operating parameters were identical.

References

1. C.E. Park et al., J. Medicinal Food, 13(4), 815-820 (2010).
2. J-G. Wu et al., J. Chem. Eng. Data, 55, 5286-5288 (2010)
3. J. Zielonka, J. Gebicki, G. Grynkiewicz, Free Radical Biology & Medicine, 35(8), 958-965 (2003)