Equivalence of High Volume Manufacturing Methods for IS CHO-CD XP™ Culture System Media

Brandy Nunez, Mark C. Arjona, Evan R. Horowitz, Anupriya Sharma, Maria Meza, and Scott D. Storms
Irvine Scientific, Santa Ana, CA, USA

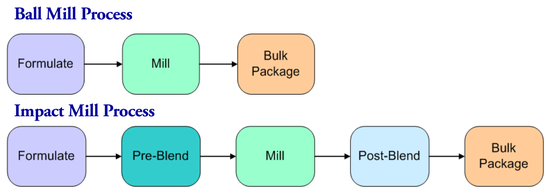
Abstract

To meet the increasing demands of the pharmaceutical industry, Irvine Scientific utilizes both impact and ball mill processes for large scale manufacture of powder media. Ball milling has traditionally been preferred, but is restricted by maximum batch size with large scale lots requiring blending of multiple ball milled sub-lots. The new impact mill process has the benefit of continuous scalability with batch size limited only by the size of the blending equipment. This process allows for more efficient manufacture characterized by greater consistency and capacity. The primary consideration during impact mill process development was product quality equivalence with the established ball mill process. A final verification was conducted to demonstrate the equivalence of the two processes using IS CHO-CD XP™ Culture System media. Six lots were made of each media and twelve samples were taken from each lot for product quality comparison. Particle size distribution, homogeneity, and standard QC tests were used for comparison of physical characteristics of each sample. A recombinant CHO cell line was cultured in the test media to evaluate cell growth performance and production. These tests demonstrated equivalence between the two processes leading to verification of the new impact mill process.

Powder Manufacturing Process Development

Irvine Scientific has developed a new powder manufacturing process utilizing an impact mill to increase manufacturing capacity and efficiency. The new process was designed to produce media powders that are equivalent in performance and product quality to those produced by the existing ball mill process even though the two methods have substantially different process flows (Figure 1). To demonstrate equivalence of the manufacturing processes, a comparison of powders from six runs of the impact mill process with powder from one ball mill run was conducted.

Figure 1. Manufacturing Process Flow.



Methods

Product Quality Assays

Twelve samples were taken from each batch of powder media according to a specific sampling plan. Particle size distribution analysis was performed on each powder sample using a Microtrac S3500 Particle size analyzer. Liquid media samples (2L for growth media, 1L for feed media) were created from each powder sample for further testing. Homogeneity was determined by statistical analysis of vitamins and amino acids in the liquid samples that were quantified using standard HPLC methodologies.

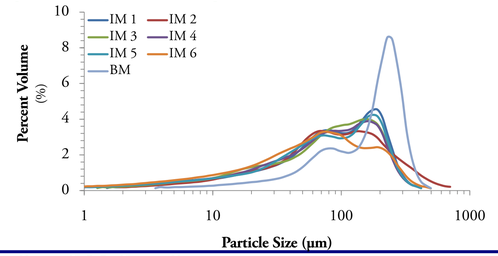
Performance Testing Using Cell Culture

A recombinant CHO cell line was used to test culture performance of the media. Cell culture was carried out in 125ml shaker flasks using standard procedures (working volume 30ml, 120 RPM agitation, 37°C, and 6% CO_2). Fed-batch cultures were grown in control growth media supplemented with a total of 12% working volume of test feed media fed on days 2 and 4 of culture. Viable cell density and percent viability of each culture were measured daily using a Vi-Cell XR™ cell viability analyzer (Beckman Coulter, USA). Cultures were terminated on day 14 and sampled for quantification of volumetric production of the recombinant protein using Protein-A affinity chromatography.

1. Particle Size Analysis of Growth Medium Powders

Particle size analysis was conducted on one sample from each growth medium manufacturing run (Figure 2). Good run-to-run consistency was seen in impact milled powders.

Figure 2. Particle Size Analysis of Growth Medium Samples.
Samples were analyzed from six impact mill (IM) and one ball mill (BM) runs.



2. Growth Medium Comparison

Equivalence between the impact and ball milled powders was demonstrated by homogeneity analysis and cell culture performance. The criterion for homogeneity was variability of <6% RSD for each component tested and all powders were homogenous (Figure 3). Cell growth, production, and viability were compared from recombinant CHO cultures (Figures 4 – 6). Culture performance was required to be ±10% of that in ball mill media and this was met in all cases.

Figure 3. Homogeneity Analysis of Growth Medium Samples.
Six impact (IM) and one ball (BM) mill runs were tested with 12 samples taken from each run. Asparagine (Asp), Threonine (Thr), Valine (Val), Niacinamide (NAA), Folate, and Vitamin B-12 (B-12) were analyzed. The red line indicates the acceptance limit of <6% RSD.

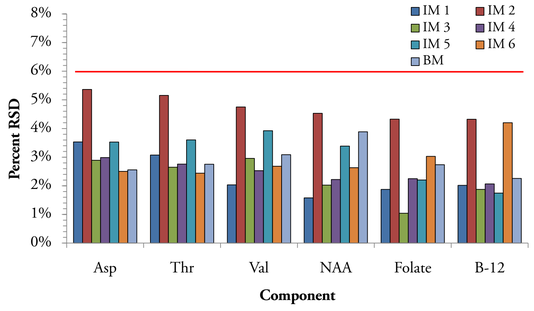
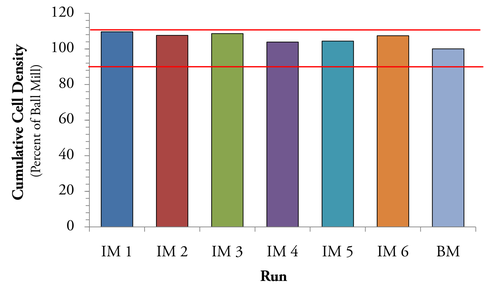


Figure 4. Cell Growth of rCHO Cultures in Growth Medium Samples.
Six impact mill (IM) runs and one ball mill (BM) run were evaluated. The red lines indicate the acceptance limits of ±10% of the ball mill (BM) control.



2. Growth Medium Comparison (cont.).

Figure 5. Production of rCHO Cultures in Growth Medium Samples. Six impact mill (IM) runs and one ball mill (BM) run were evaluated. The red lines indicate the acceptance limits of ±10% of the ball mill (BM) control.

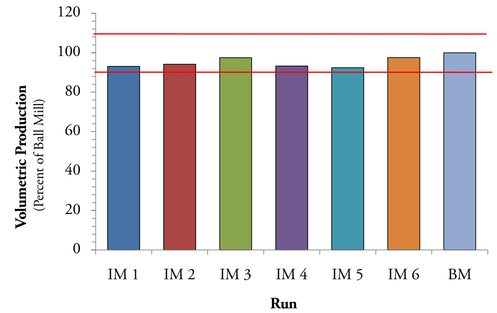
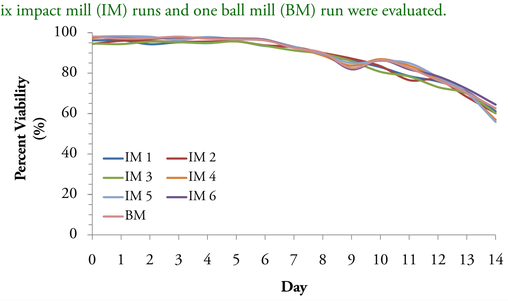


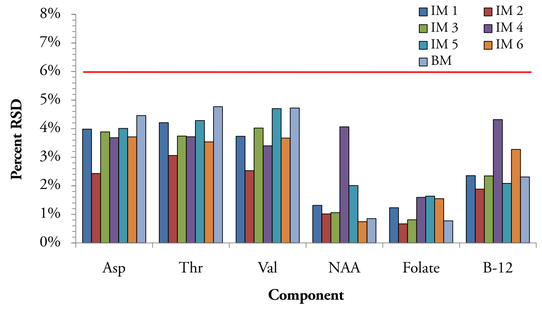
Figure 6. Viability of rCHO Cultures in Growth Medium Samples.
Six impact mill (IM) runs and one ball mill (BM) run were evaluated.



3. Feed Medium Comparison

The methods to demonstrate equivalence between the impact and ball mill manufacturing processes for the feed media were the same as the growth media (Section 2). For feed media experiments, cell culture was run in fed-batch mode (feeding a total of 12% working volume over two days) for each feed sample using a common growth medium for all cultures. The criteria for homogeneity, cell growth, production and viability were met for all samples so the manufacturing methods are deemed equivalent for feed media (Figures 7-10).

Figure 7. Homogeneity Analysis of Feed Medium Samples.
Six impact (IM) and one ball (BM) mill runs were tested with 12 samples taken from each run. Asparagine (Asp), Threonine (Thr), Valine (Val), Niacinamide (NAA), Folate, and Vitamin B-12 (B-12) were analyzed. The red line indicates the acceptance limit of <6% RSD.



3. Feed Medium Comparison (cont.).

Figure 8. Cell Growth of rCHO Cultures in Feed Medium Samples. Six impact mill (IM) runs and one ball mill (BM) run were evaluated. The red lines indicate the acceptance limits of ±10% of the ball mill (BM) control.

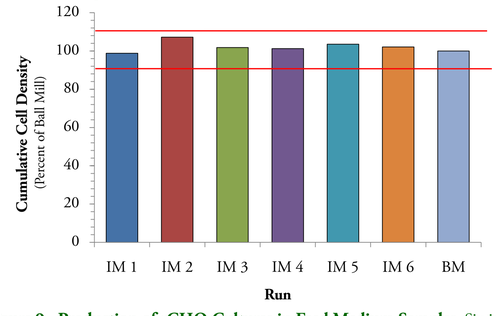


Figure 9. Production of rCHO Cultures in Feed Medium Samples. Six impact mill (IM) runs and one ball mill (BM) run were evaluated. The red lines indicate the acceptance limits of ±10% of the ball mill (BM) control.

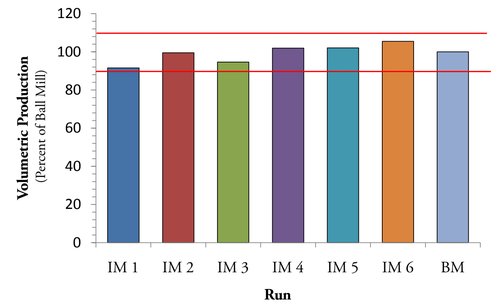
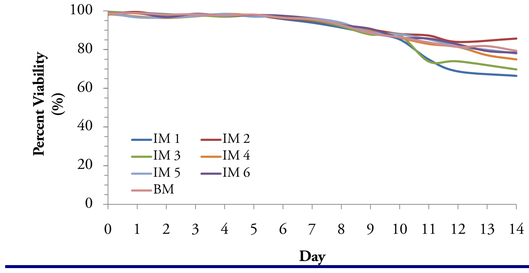


Figure 10. Viability of rCHO Cultures in Feed Medium Samples.
Six impact mill (IM) runs and one ball mill (BM) run were evaluated.



Summary & Conclusions

Impact and ball mill processes produce powders with equivalent quality and performance for IS CHO-CD XP media.

1. Particle size distribution was slightly different between powders from the two processes but had no impact on product quality.
2. Homogeneity and cell culture performance were consistent and statistically equivalent between media made using either method.
3. Impact milling provides an effective and reproducible process for media manufacture.

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