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Pharma & Biopharma Raw Material Solutions

Technical Brief

Enabling Process Intensification with Compacted Cell Culture Media

Summary

Compacted cell culture media (CCM), such as the EZMix™ custom CCM product line, enables intensification of modern biopharmaceutical manufacturing processes. Compacted CCM offers significantly faster dissolution times and reduced packaging volume compared to existing fine powders. The enhanced flowability and minimal dust formation protects employee health and safety, and simplifies the transfer of material into production vessels. Most importantly, compacted CCM delivers excellent performance and quality consistent with fine powdered CCM. Taken together, these comparative advantages will allow you to increase plant throughput and flexibility, reduce facility footprint, and improve operator safety in your upstream process.

This technical brief summarizes the process of creating compacted CCM granules, as well as studies evaluating their handling efficiency and cell culture performance. Compaction data show that this new technology can help overcome the difficulties of using dry powder CCM in bioprocessing. The results have been obtained using the same Cellvento® catalogue cell culture media and feeds in powder and compacted form. This can be extended to any EZMix™ custom formulation.

Introduction

Media preparation for perfusion cell culture processes can be a challenge when intensifying upstream monoclonal antibody (mAb) manufacturing. In comparison to batch processes where media volume is roughly equivalent to the bioreactor volume, a comparable perfusion process requires media volumes up to 4x that of the bioreactor volume. At high volumes, media preparation becomes the main bottleneck with regards to time, safety, and quality. As a result, media preparation and consumption are the main focus when evaluating operational costs, storage footprint, and upstream flexibility.

Dry powder CCM offers many advantages with respect to shipping and storage due to reduced volume and increased stability compared to liquid CCM. However, such media need to be highly soluble and easy to handle. This can be achieved by dry granulation, the agglomeration of fine CCM powders into larger granules using compression force. The EZMix™ CCM product line offers advantages for biopharma manufacturers looking to enhance the efficiency of their upstream processes and eliminate bottlenecks in production. Importantly, these positive effects do not impact cell culture performance, as the only difference between dry powder and granulated CCM is in physical format not formulation.

The Granulation Process

Roller compaction is a dry granulation technique that applies compression force to produce granules from a fine powder without the need of additional water or excipients. Because the powder is exposed to less heat as compared to wet granulation, which requires a drying step, media composition of the resulting granules remains the same. In the process of roller compaction, the main parameters are compression force and mill grid size (**Figure 1**).

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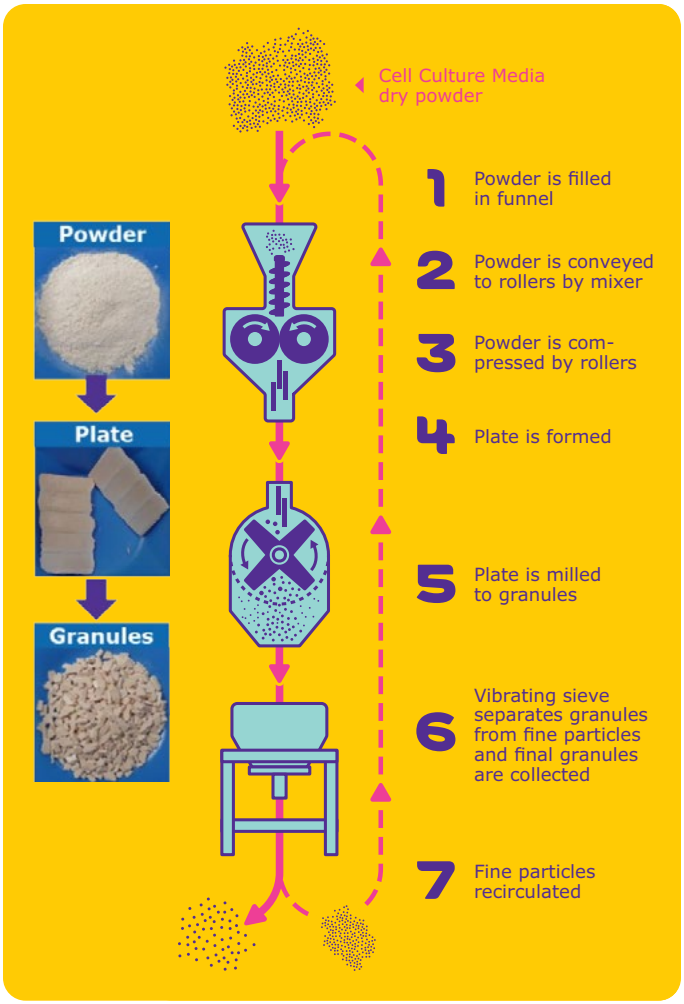


Figure 1. Flow chart of roller compaction technology used for cell culture media powders. The powder is filled in the funnel (1). By means of a mixer and a tamp auger, the powder is conveyed to the rollers (2). The powder is compressed in between the rollers by hydraulic pressure (3) forming a plate (4). The plate is milled to granules by a rotor mill (5). Non-compacted fines are separated from granules by a vibrating sieve, and final granules are collected (6). Fine particles are recirculated back into the funnel to increase final yield (7).

Consistent Cell Culture Performance and Quality

Compacted CCM must have the same cell culture properties as the fine powder. To verify this, the concentrations of selected CCM raw materials within the formulation were analyzed. A comparison of fine powder to compacted media and feed shows no difference in CHO cell culture performance and critical quality attributes.

Cell culture performance, as measured by viable cell density (VCD), viability, and product titer, was obtained with both powder and compacted media and feed (**Figure 2**). Critical quality attributes, such as aggregation and glycosylation of a monoclonal antibody, were equivalent in both conditions (**Figure 3**). No impact on pH, osmolality, and solubility was observed (**Figure 4**) and the distribution of raw materials, including vitamins, amino acids, and trace elements, in the powder and compacted media was homogeneous (**Figure 5**).

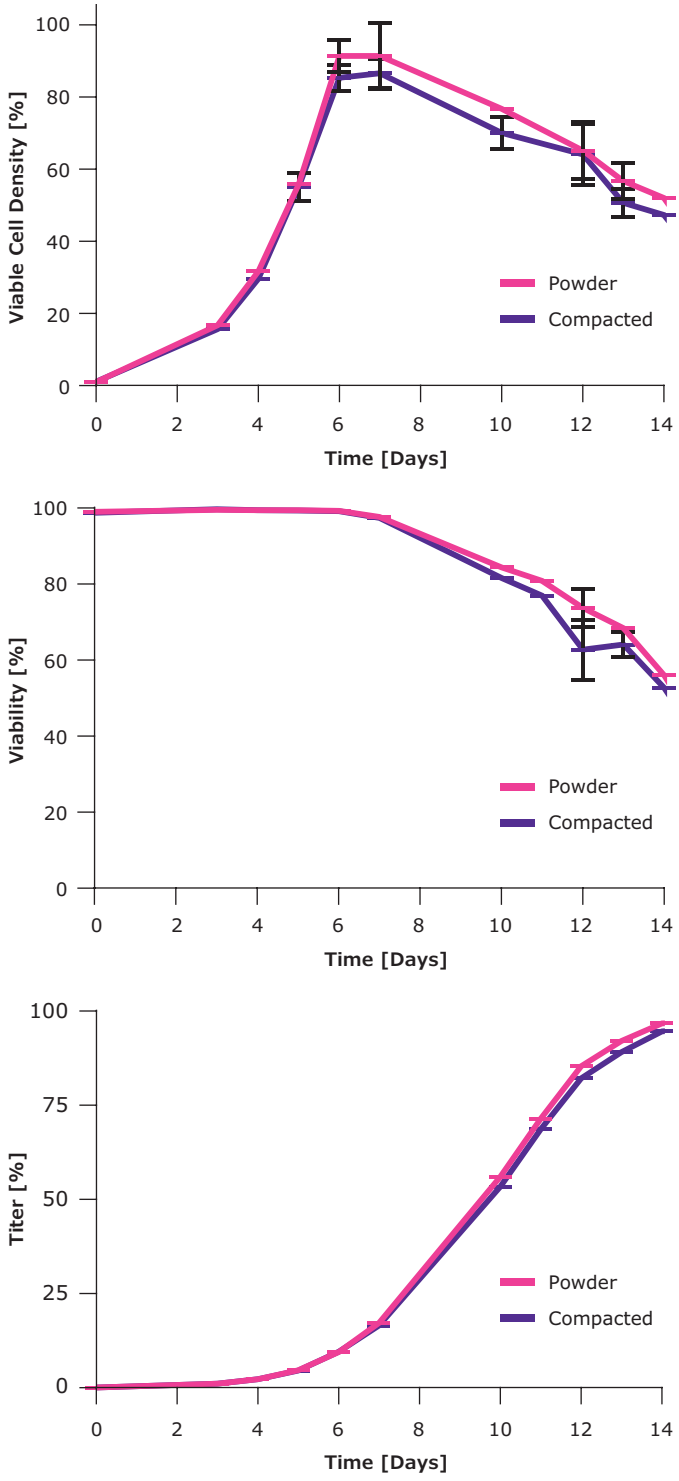


Figure 2. Similar trends in viable cell density (VCD), viability, and product titer with either the powder or compacted forms of medium and feed. Four biological replicates were performed per condition.

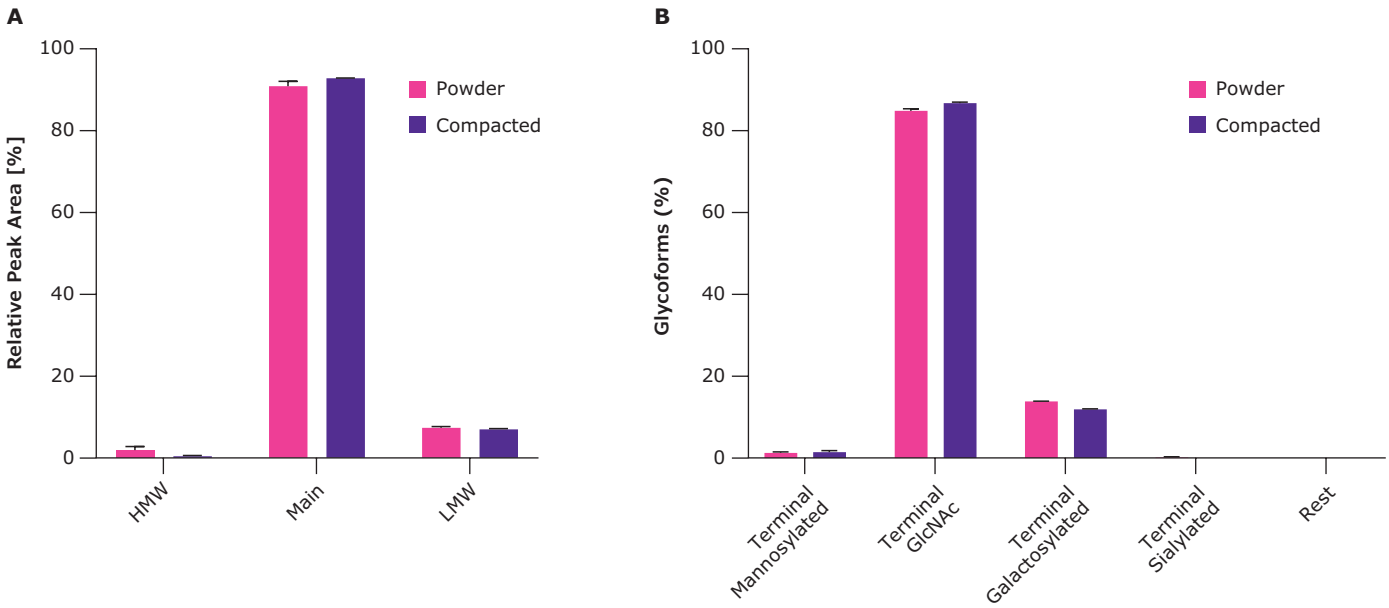


Figure 3. Similar aggregation and glycosylation patterns observed with either the powder or compacted forms of medium and feed. (A) Similar levels of high molecular weight (HMW), low molecular weight (LMW), and intact antibody (Main) observed for both powder and compacted medium and feed. (B) Various glycosylated forms of a monoclonal antibody produced by a CHO cell line, including terminal mannose, terminal N-Acetylglucosamine (GlcNAc), terminal galactose, and terminal sialic acid, have similar levels in both powder and compacted medium and feed.

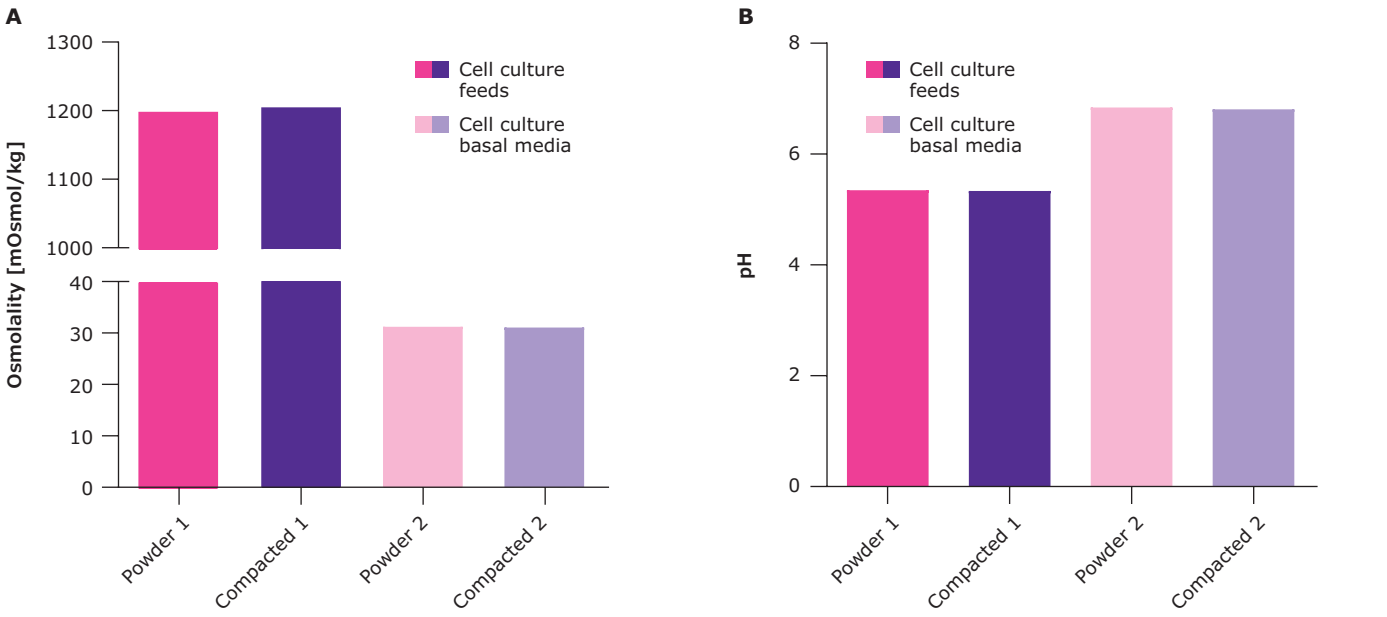


Figure 4. No difference in osmolality (A) or pH (B) with either the powder or compacted forms of cell culture medium and feed.

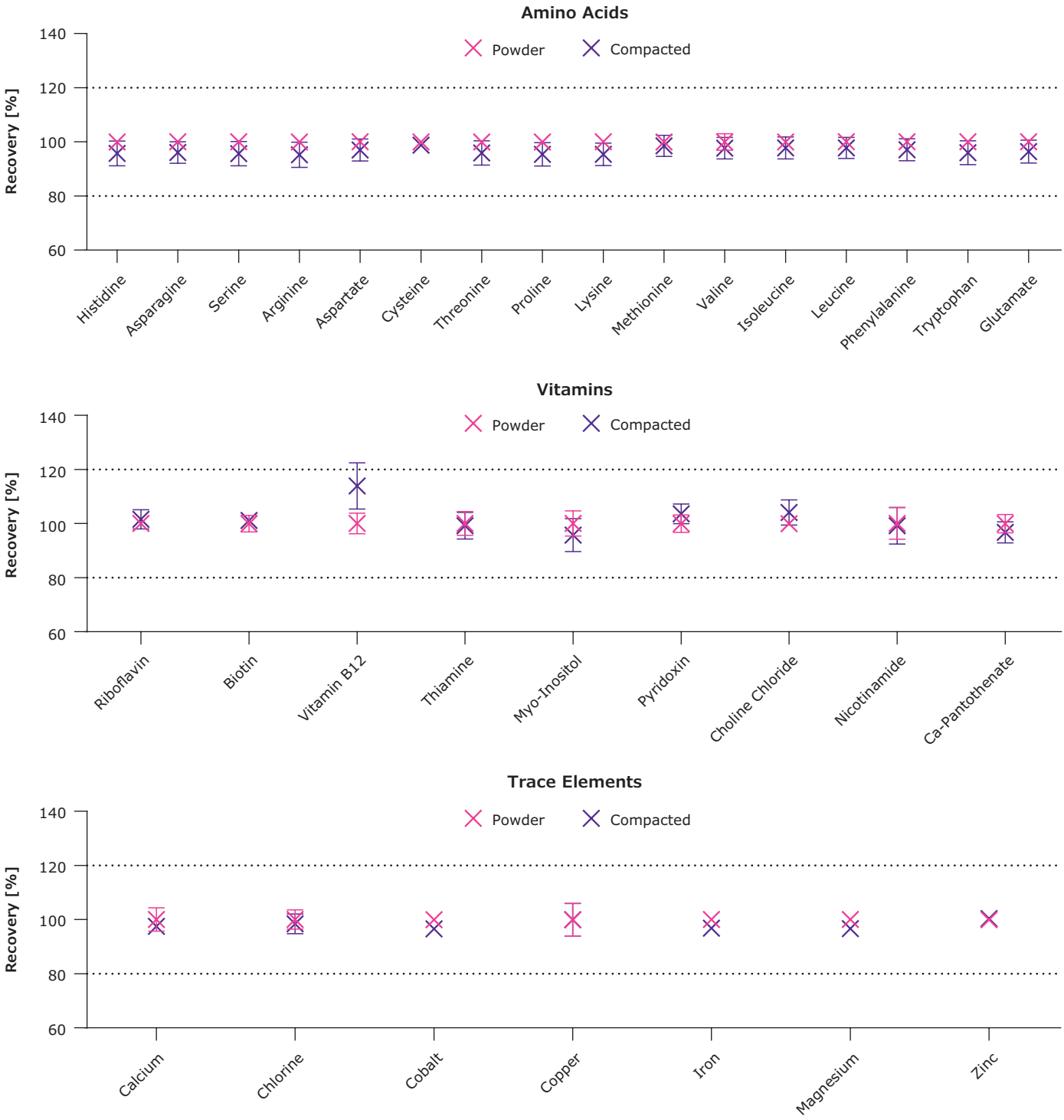


Figure 5. Equivalent levels of amino acids, vitamins and trace elements observed with either the powder or compacted form of feed. Samples taken from the beginning, middle, and end of production of powder and compacted feed are shown as Mean ± Standard Deviation. Recovery values for all components fall between 80–120%, indicating that compaction does not lead to depletion or degradation of components.

Optimized Dissolution Kinetics

Optimizing dissolution times can simplify and streamline media preparation, especially when large volumes of media are required for perfusion processes, or when dealing with less soluble, highly concentrated media formulations. With compacted cell culture media and feeds, hydration time is significantly decreased as shown in **Table 1** and **Figures 6** and **7**. The time required to hydrate compacted CCM can be reduced two- to six-fold in magnitude, depending on vessel size and addition method (e.g., powder transfer bags vs. shoveling). It should be noted that the reduction in dissolution time depends on the media formulation as well; the effect is more pronounced for cell culture feeds compared to cell culture basal media.

Table 1. Decreasing hydration time of compacted cell culture feed in a 1000 L single-use mixer.

	Powder	Compacted	t_{Powder}/t_{COMP}*
Time to add first 12.93 kg (by powder transfer bag)	120 s	20 s	**6x**
Time for addition of remaining 112.93 kg (by shoveling from container)	50 min	23 min	**2x**
Time after end of addition until turbidity < 3 NTU	33 min	18 min	**2x**
Total hydration time [addition + dissolution < 3 NTU]	83 min	42 min	**2x**
Summary	2- to 6-fold difference in time attributed to shoveling compared to powder transfer bag addition		

*Fold more time needed for powder

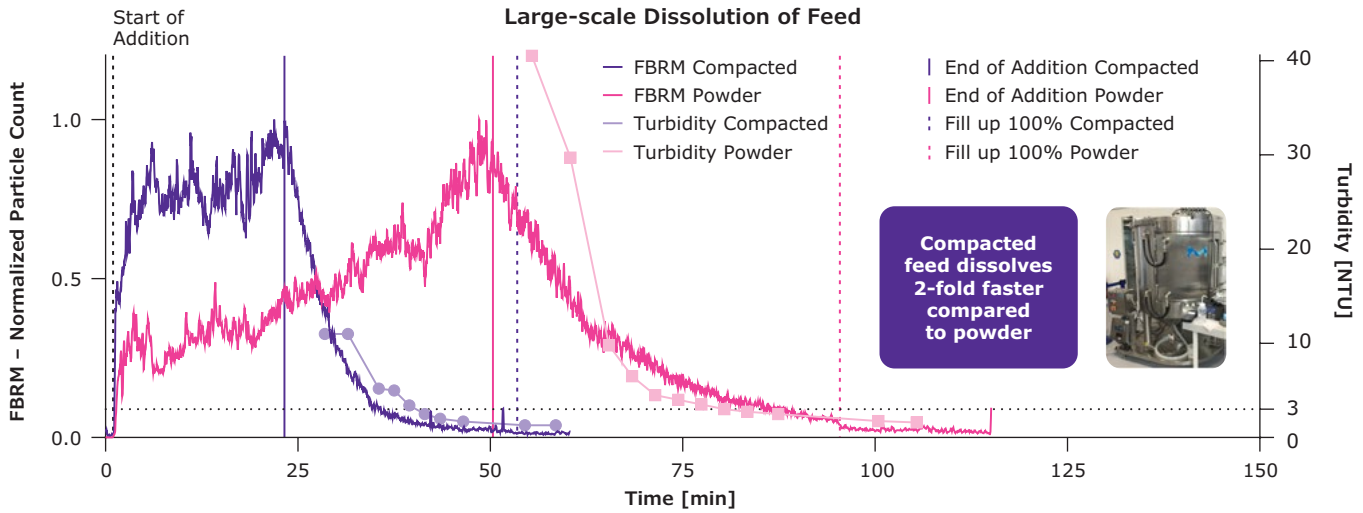


Figure 6. In a large-scale mixer, hydration time decreases by 2-fold using a compacted feed compared to powder, leading to process intensification. Equivalent concentrations of powder and compacted feed were added to the Mobius® Power MIX 1000 L mixer. Focused Beam Reflectance Measurement (FBRM) was used to count particles in solution, with decreasing particles indicating a fully dissolved solution. Turbidity was measured externally from samples taken from the mixer. Decreasing turbidity represents dissolution of particles, and values below 3 NTU indicate full dissolution.

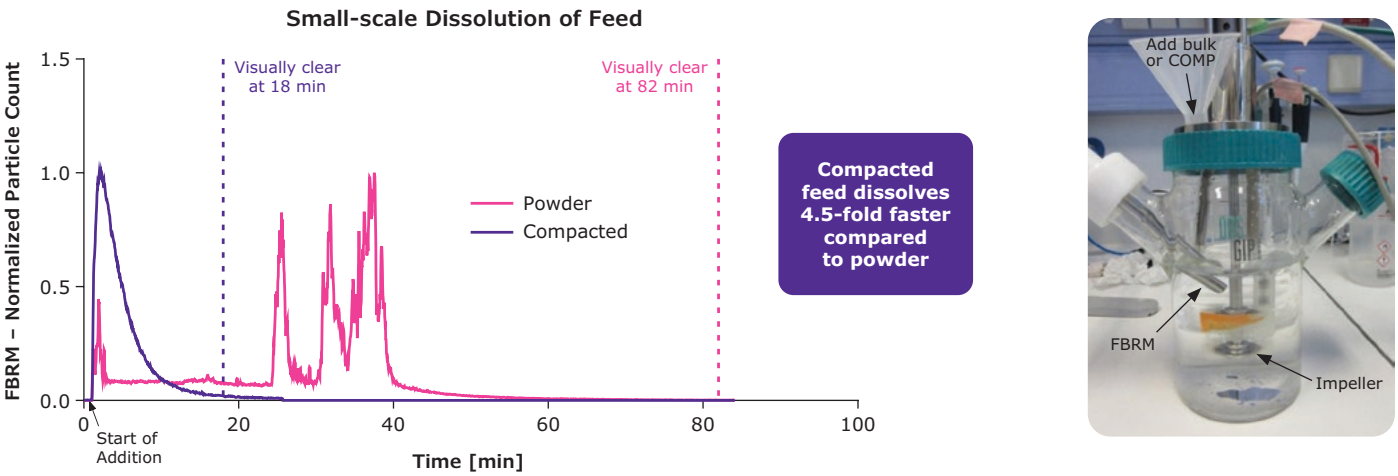


Figure 7. In a small-scale bioreactor, hydration time decreases by 4.5-fold using a compacted feed compared to powder, leading to process intensification. Equivalent concentrations of powder and compacted feed were added to an 800 mL bioreactor. Focused Beam Reflectance Measurement (FBRM) was used to count particles in solution, with decreasing particles indicating a fully dissolved solution. Turbidity was measured externally from samples taken from the bioreactor. Final turbidity for powder (1.46 NTU) and for compacted media (1.88 NTU) were below 3 NTU, indicating full dissolution.

Bulk Volume Reduction

Compared to dry powder CCM, compacted CCM offer advantages that can improve shipping efficiency and risk mitigation in the media preparation process.

Our data suggest footprint savings using compacted CCM of up to 46% of the space required for fine powder media (**Figure 8**). The degree of bulk volume reduction depends on the media formulation with the impact being greatest for cell culture feeds (Powder 1, Compacted 1) compared to cell culture basal media (Powder 2, Compacted 2). Consequently, the smaller bulk volume of compacted cell culture media compared to fine powder cell culture media equates to a significant reduction in the transport and storage area required in the manufacturing plant.

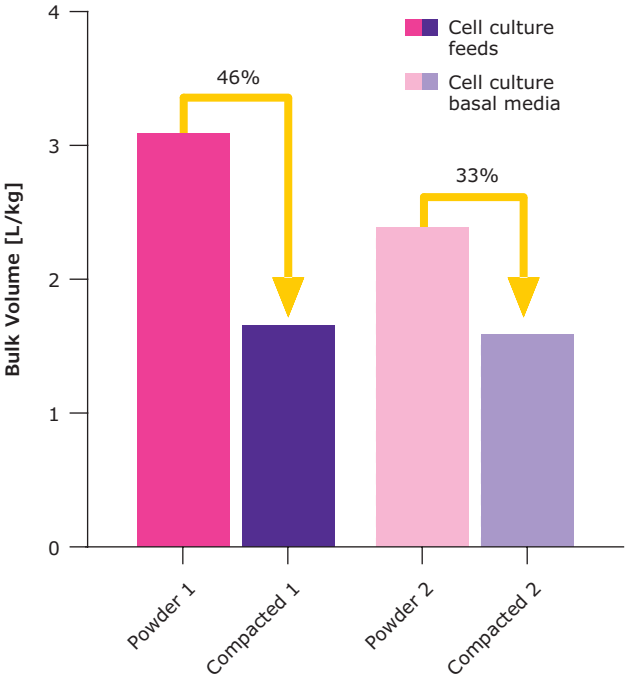


Figure 8. Compaction reduces bulk volume of cell culture medium and feed. Bulk volume was measured after adding 20 g of powder and compacted media into a measuring cylinder.

Dust Reduction

Handling and use of powdered cell culture media can lead to dust formation, which is a major health and safety concern and requires protective measures. Dust is also a potential contamination source.

Use of compacted CCM results in decreased dust formation during handling (**Figure 9**). Dust reduction leads to lower risk of bioburden contamination and fewer cleaning requirements. More importantly, employee safety is increased due to reduced product dust inhalation and a decreased likelihood for creation of an explosive dust atmostphere.

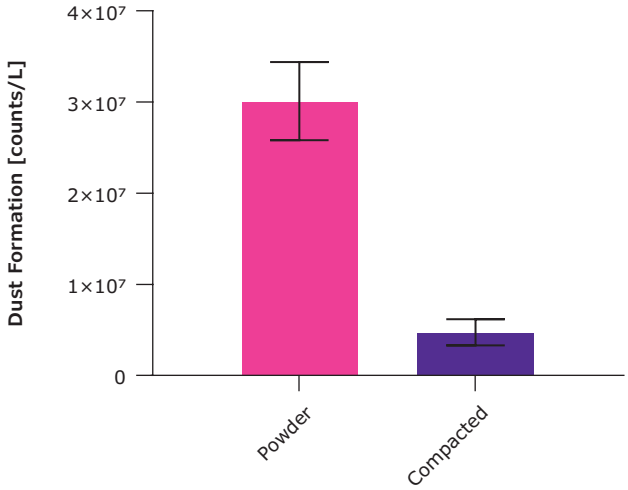
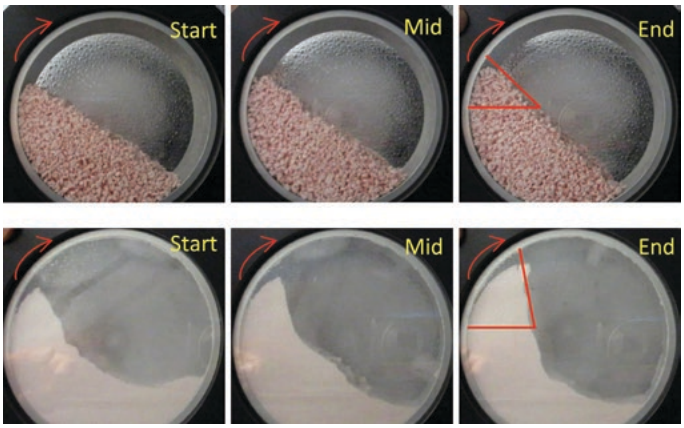


Figure 9. Compaction significantly reduces dust formation for cell culture media and feed. An aerosol spectrometer was used to measure 3 replicates of dust fractions from 0.265–34 µm while pouring powder or compacted medium into a hydration vessel.

Improved Flowability

The superior flowability of compacted media results in improved handling during weighing and dispensing into the hydration vessel (**Figure 10**). Improved flowability also decreases dust formation because the material flows in a consistent manner. The compacted cell culture medium does not stack up like fine powder would, and therefore does not fall uncontrolled into the vessel.



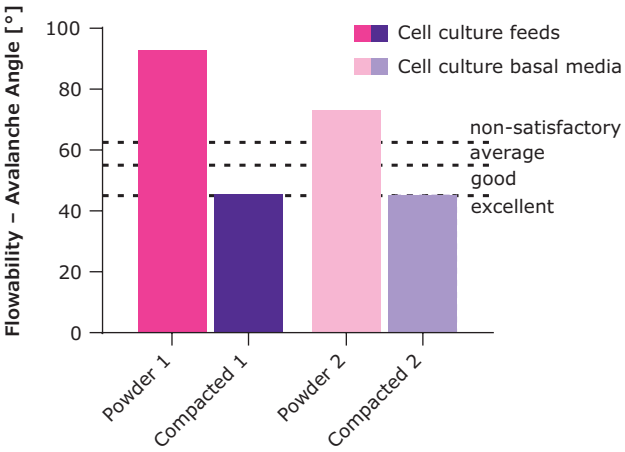


Figure 10. Compaction improves flowability of cell culture medium and feed. The video of the rotating powder or compacted cell culture medium was analyzed. The avalanche angle of the rotating material is measured at the top left of the drum. Decreasing angles correspond with better flowability of the CCM. The effect is more pronounced for cell culture feeds (Powder 1, Compacted 1) compared to cell culture basal media (Powder 2, Compacted 2).

Process Economics

Improvements in dissolution kinetics, bulk volume, dust formation, and flowability reduce process costs and improve operator safety. Faster dissolution reduces labor time, allowing operators to focus on value-adding activities. In addition, higher bulk density means that the same volume of media can be packaged into fewer containers, reducing incoming quality control testing costs and freeing up valuable freezer storage space. Lastly, reduced dust formation and improved flowability help create a safer environment for operators and reduce the risk of costly employee health and safety incidents.

More importantly, compacted CCM enables transformative technologies such as single-use mixing and media concentrates. These technologies can add value to any process, but they are especially impactful for media-intensive manufacturing processes, including perfusion and intensified batch. The improved dissolution kinetics of compacted CCM can help to overcome limitations in the fluid dynamics of single-use mixers, allowing users to experience enhanced flexibility and time savings. In addition, compaction decreases the dissolution time of media concentrates, making their use a feasible option for processes otherwise excluded by long mix times and bioburden concerns. Concentrates, in turn, can save money by reducing the size of single-use prefilters and storage bags. Together, these technologies can lead to significant cost savings with each manufacturing run, as can be proven by total cost of ownership models.

Conclusion

Compacted CCM delivers an equivalent performance to powder media and offers important advantages for intensified upstream processes:

- **Faster dissolution** accelerates the upstream workflow for biopharma manufacturers aiming to gain operational flexibility and efficiency by process intensification.
- **Increased flowability** leads to easier handling and **reduced dust formation**, improving employee safety and minimizing the risk of contamination.
- **A smaller bulk volume** reduces storage requirements and space needed for transportation.

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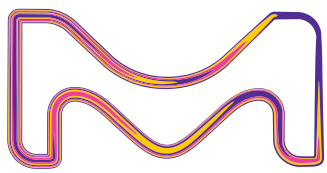

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