A NOVEL APPLICATION OF GRANULATION TECHNOLOGY TO IMPROVE PHYSICAL PROPERTIES AND BIOLOGICAL PERFORMANCE OF POWDERED SERUM-FREE CULTURE MEDIA.

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Abstract

The Advanced Granulation Technology (AGT) process was evaluated using serum-free and protein-free formulations containing ingredients believed to be sensitive to the ball-milling process. The AGT manufacturing process was scalable, based upon physical parameters, biochemical homogeneity and biological performance criteria. Cell culture growth and biological production studies involving CHO, Vero, hybridoma and HEK 293 cells demonstrated that serum-free/protein-free media derived from the AGT process supported equivalent or superior performance to identical formulations produced by traditional processes. AGT meets pharmaceutical quality guidelines and can be applied to a broad range of nutrient formulations used in bioproduction applications.

1. Introduction

Dry powdered medium (DPM) historically has been manufactured using ball-milling technology [1]. Constituents of the nutrient formulation (e.g., amino acids, salts, vitamins, carbohydrates, etc.) are simultaneously crushed and mixed within a large, ceramic mill containing grinding stones of various sizes, shapes and composition. Alternative methods for particle attrition (e.g., jet milling, hammer milling) have alleviated some problems associated with the ball milling process. However, critical limitations of the basic powder format persist, such as:

- Cross-contamination and health concerns associated with dust generation
- Lengthy and incomplete dissolution/solubilization processes
- Requirement to segregate "sensitive" ingredients from the basal formulation and to add them separately to the hydrated powder
- Homogeneous distribution of minute formulation components
- Requirement for post-formulation pH and osmolality adjustment

We applied the well-characterized pharmaceutical technology of fluid bed granulation to nutrient medium manufacturing [2-4]. Advanced Granulation Technology (AGT) consists of spraying aqueous solutions of concentrated medium components onto air-suspended attrited powdered medium components within a stainless steel chamber. Water is rapidly evaporated under controlled conditions, resulting in complete and

homogeneous distribution of nutrient ingredients throughout the resultant aggregated granules.

2. Materials and Methods

Dry-form media were prepared by multiple methods using identical biochemical constituents for selected serum-free or protein-free specialty formulations. Control media were manufactured by conventional ball-milling procedures or by direct weighing of identical constituents, then supplemented, as required, by individually-weighed ingredients or liquid concentrates for constituents considered sensitive to ball-milling (Figure 1A). AGT-based media incorporated all formulation constituents into granules (Figure 1B).

Figure 1A. Traditional Media Preparation

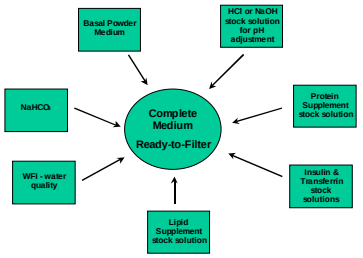
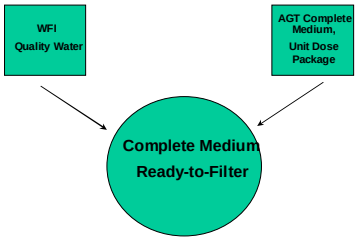


Figure 1B. AGT Medium Preparation



Control and AGT media were reconstituted to liquid formulations as described above, sterilized by membrane filtration, and evaluated for biochemical and biological performance parameters. Kinetics of medium dissolution were observed at the 7 L formulation scale (room temperature) and monitored by digital photography. Replicate samples for analysis of intralot homogeneity were taken from representative locations throughout each production lot, encompassing initial, middle and final processing phases. Analysis of pH and osmolality was performed by classical methods. Content of amino acids, vitamins and other medium components was determined by HPLC. Cell proliferation and biological production assays unique to the particular cell type, target product and specialty medium combination have been described elsewhere [5-6].

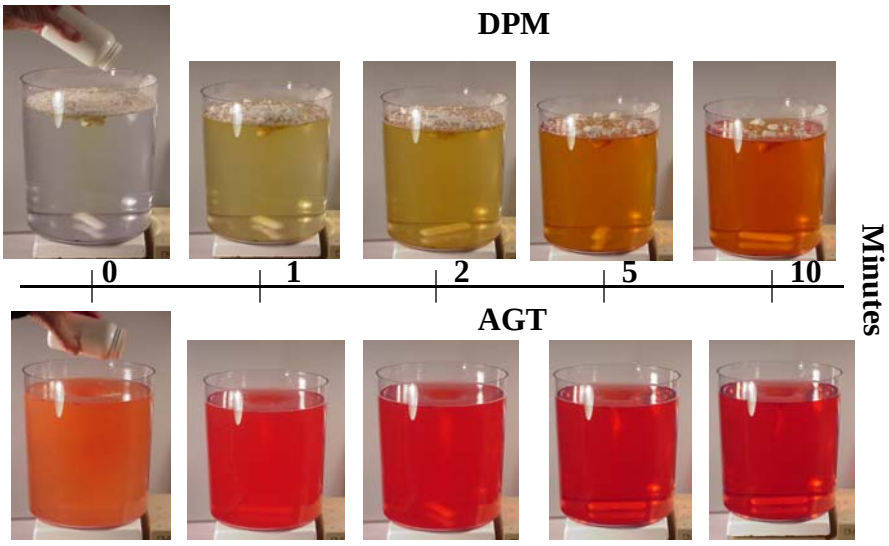
3. Results

3.1. Dissolution Kinetics

Figure 2 compares the rate of dissolution of DPM (top panel) and AGT (bottom panel) versions of VP-SFM, a serum-free specialty medium designed for virus production applications. For DPM, the zero time photograph illustrates the initial layering of medium constituents and subsequent dissolution over the 10-minute time course. The DPM-based formulation was not fully clarified (by visual inspection and osmolality, data not shown) for approximately 30 minutes. By contrast, the AGT granules were rapidly hydrated and dispersed at the zero time point and complete solubilization was noted by

the 10-minute time point. (Note: The gradual color change with the DPM sample corresponds to the dissolution of biochemicals intrinsic to the powdered format. Sodium bicarbonate and other required additives were added separately during medium constitution. Sodium bicarbonate and other constituents were already incorporated into the AGT granules (see Figure 1); thus, no color change was expected or observed).

Figure 2. Comparative Dissolution Kinetics



3.2. Homogeneity and Scalability

A fundamental process qualification issue concerned the homogeneous distribution of constituent biochemicals by the granulation process. Replicate samples were analyzed from representative locations throughout pilot production batches and from multiple lots. Samples were evaluated for pH and osmolality and for amino acid and vitamin content. Homogeneity was evaluated statistically, using two-way analysis of variance (ANOVA) with a 0.05 level of significance. No statistical difference was observed for any of the tested parameters.

We analyzed the ability of specialty formulations produced by the granulation (AGT) process to support biological performance. Figure 3 compares growth and β-galactosidase expression for a CHO cell transfectant adapted to grow in CD CHO, a protein-free, chemically-defined specialty medium designed for CHO cell-based recombinant protein expression applications. Vero cell growth and production of relevant viruses are illustrated for AGT-derived VP-SFM (Figure 4). Biological performance of these AGT-based media, and for other catalog and custom formulations, was equivalent to identical formulations produced by conventional methods.

Figure 3. CHO Transfectant Expression CD CHO (AGT Format)

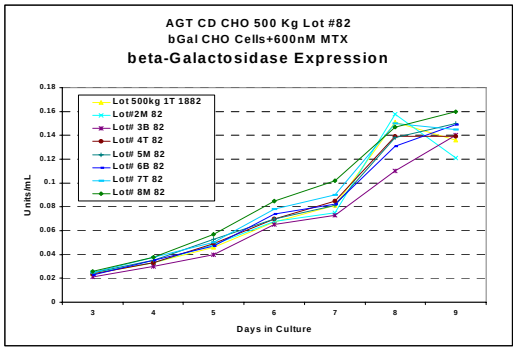
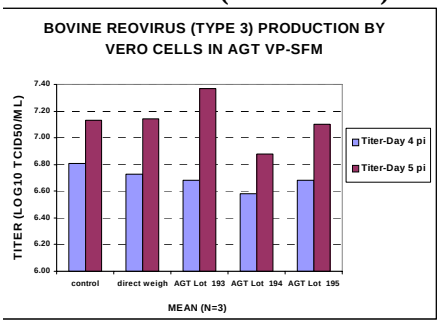


Figure 4. Virus Expression AGT VP SFM (AGT Format)



4. Discussion

4.1. Challenges of Serum-Free and Protein-Free Media in Dry Format

While standard media have been successfully formulated as ball-milled powders for decades [1], the diverse composition of novel serum-free and protein-free media presents unique challenges. Some constituents (e.g., bioactive peptides, lipids) are difficult to introduce into homogeneous powders and to recover quantitatively during hydration and subsequent sterile filtration. These and other biochemicals can be sensitive to the physical stresses and heat generated by the ball-milling process. We have reported [7] diminished biological performance for DPM compared with identical formulations prepared from liquid concentrated intermediates, and have consequently chosen not to commercialize certain media in the DPM format. Use of the AGT process enables us to supply these formulations in dry (granular) format with performance equivalent to freshly-prepared liquid media produced by direct weighing of biochemicals or by reconstitution of liquid concentrated intermediates.

Specialty media designed for biological and vaccine production applications involving mammalian cells were produced by the AGT process at pilot (9 Kg and 90 Kg) and production (500 Kg) scales and compared with identical formulations manufactured by conventional technology. We demonstrated that AGT product homogeneity and that the AGT production process was scalable over this range. Batch sizes of AGT media may be further increased through conventional blending techniques.

4.2. Dissolution and Solubilization

A frequent complaint regarding DPM is the protracted dissolution time and incomplete solubility. The requirement to manufacture complex formulations as a basal DPM plus multiple additives also creates procurement, quality assurance and inventory management complexity and increases the potential for misformulation errors. Use of complete media supplied in the AGT format apparently alleviates many of these concerns. Figure 1 illustrates the reduced complexity of maintaining raw material inventory and of reconstituting nutrient medium from AGT granules. Dry-form medium in this granular physical state generates significantly less dust and hydrates instantly when added to water. All required nutrient components of a complete formulation (including sodium bicarbonate, lipids, hydrolysates, peptide growth factors, trace metals, etc.) may be incorporated into these homogeneous granules, eliminating the need to qualify and formulate these components separately. Nutrient medium supplied in the AGT format dissolves rapidly into production water with no requirement for subsequent manual pH or osmolality titration.

AGT-based medium (Figure 2) dissolves more rapidly and completely in formulation water, obviating the need for temperature elevation, strenuous agitation, rapid dissolution equipment, or extended mixing times. Statistical homogeneity analyses document the quantitative consistency of measured analytes within production batches and during manufacturing scale-up qualification. AGT-based specialty media for recombinant protein (Figure 3) and for virus production (Figure 4) and other applications (manuscript in preparation) exhibited equivalent biological performance when compared with control media produced by traditional formulation methods.

5. Conclusion

Advanced Granulation Technology (AGT) is an effective, pharmaceutical quality process for manufacturing serum-free and protein-free specialty media in dry format. Implementation of AGT-based media may substantially reduce total cycle cost and resource requirements for industrial scale formulation of complex media.

6. References

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