

Technical Bulletin

Particle Size Analysis in Dry Powder Cell Culture Media Production

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Introduction

Product testing is constantly evolving as underlying technologies improve. Particle size determination of comminuted powders – a vital element in dry powder cell culture media production – has existed in various forms for centuries, but modern improvements in the accuracy, range and reporting abilities of particle size analyzers (PSA) have extended our capabilities significantly. With the added accuracy and ease of use that comes with PSA equipment, the trend has been to incorporate these results into product specifications. Do these specifications really add value if there is no scientific backing of the results?

PSA alone is not sufficient to fully represent the physical characteristics of a complex media. It is the empirical evidence of particle size and the effects of changes in particle size distribution which are important tools in assessing the handling properties of milled powder. This paper offers an overview of the particle size measurement capabilities of SAFC Biosciences®; the effects of particle size variability; ways to communicate the distribution in a beneficial manner; and how best to use this powder characterizing tool. The goal is to demonstrate how SAFC Biosciences uses current technologies in particle size analysis to provide a consistent, high-performing media.

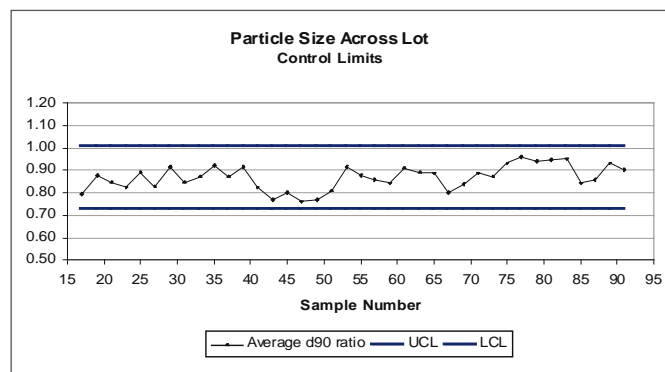
Particle Size Analysis Capabilities

Currently, SAFC Biosciences uses a Ro-tap® sieve sifter for creating physically separated streams based on product classification through mesh screens. The Ro-tap sifter results have value, but the physical screening action itself can cause particle size reduction and agglomeration of fine particles make it

unreliable below 75 microns.^[1] As impact milling can produce fines in the sub-micron range another method must be used to characterize the particle size distribution.

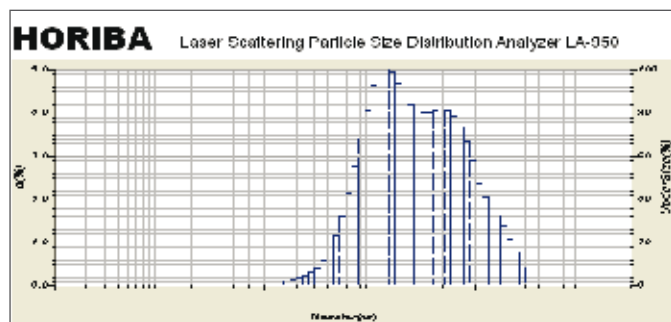
SAFC Biosciences also uses a Horiba LA-950 laser diffraction analyzer. This instrument uses Mie scattering theory of edge and particle diffraction to calculate particle size from 10 nm to 3000 μm .^[2] A computer algorithm provides a curve of particle diameters. Results can be communicated through statistical or graphical means. The Horiba LA-950 does not determine particle shape and assumes all particles to be spherical. This assumption is critical as particle shape affects the results, as well as powder behavior.

The Horiba particle size analyzer requires approximately 1.5 grams of material. Of those 1.5 grams, only a portion is measured. This small test size leads to variation among samples. To minimize sampling and testing errors, proper representative sampling techniques are used and tests are performed in replicate. Variability between samples is compared to assess variation across an entire lot.



Particle Size Statistical Analysis

Since particle size for a complex media is a distribution of diameters, statistics can be used to convey the results. A common method is to use d10, d50 and d90 values based on volume distribution. That is to say that 10%, 50% and 90%, respectively, of the particle size distribution is smaller than the stated diameter. Standard deviations and span calculations are also appropriate when discussing the distribution width. The calculated surface area also provides a helpful frame of reference for solubility conditions. Communicating particle size distribution through statistic expressions alone can fail to report important information such as bimodality, which can be conveyed through graphical means.



Example of Horiba LA-950 Result

Different measurement techniques call for different means of expression, but independent of the method, it is critical to realize one particle size setpoint will be of low value. Reporting a d90 will give an idea of the upper diameter range, it will not convey the amount of fine particles in the sample. Operationally, this might lead to manipulation of the milling system to meet a value that has little meaning. For instance, by increasing the mill speed and obliterating the particles you would meet a d90 specification, but the resulting powder would have been exposed to higher temperatures and may have a dramatic increase in sub-micron fine particles.

Responses to Particle Size Variations

The effects of particle size and distribution changes are multiple and no single parameter can be used to fully define the powder. The following aspects are inter-dependent and all have a major impact on physical properties:

- angle of repose
- flowability
- bulk density
- particle size and distribution
- particle shape
- cohesiveness
- adhesiveness
- agglomeration
- friability
- abrasiveness
- material composition
- surface characteristics
- moisture content
- density
- viscosity

Most of these properties are inherent to the components of the formulation and cannot be changed. For this paper, three significant areas of concern related to particle size will be discussed: material handling properties, mixing/segregation and solubilization.

Material handling properties - During manufacturing, the material will show differences in handling as the particle size changes. These properties are component dependent. Assuming a recipe is held constant there are several variables that can be affected by particle size. Material handling attributes are hard to define, but practical experience has provided some tests. The terms flowability and floodability are empirical values normally associated with the Carr indices. The Carr indices include density measurements, angles of repose, and compressibility. Through these evaluations, the ease of handling for the powder can be predicted and engineering solutions can be created.

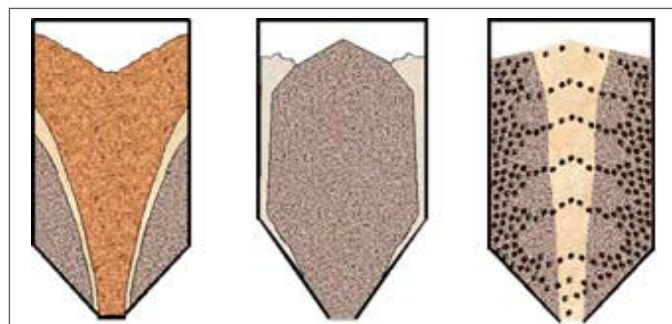
SAFC Biosciences performs Hausner ratio tests to predict flowability for the finished powder. The Hausner ratio is the ratio of the tapped density over the free settled bulk density.^[3] The general rule is that Hausner ratios greater than 1.25 indicate that the material will flow poorly. For most cell culture media the free bulk

density decreases as particle size decreases as a result of entrained air between the fine particles. As such it is beneficial to keep overall particle size distribution as high as possible without putting the material at risk for sifting segregation or poor solubility. These complexities show the problems that may result from establishing an artificially defined maximum particle size. Product milled to ultra-fine particle sizes will be difficult to pour from drums or barrels, create a dusty formulation environment and can require additional design considerations for proper material handling.

Relationship between Hausner ratio and Flowability	
Hausner ratio	Flow Character
1-1.11	Excellent
1.12-1.18	Good
1.19-1.25	Fair
1.26-1.34	Passable
1.35-1.45	Poor
1.46-1.59	Very poor
>1.6	Non-flowing

Mixing/segregation - Particle size has implications for mixing/demixing properties. Since particle size affects flow properties, the ability to achieve homogeneous blends is also affected. Research has shown that there is a direct correlation between the rate and degree of mixing and flowability.^[5]

The converse of mixing is segregation and all mixed powders have a certain amount of likelihood to segregate. This can be minimized through manipulation of the material, optimizing the process and improving equipment design. By changing particle size distribution the tendency of a product to segregate can be reduced. Sifting segregation is the movement of smaller particles through a mixture of larger particles. Experiments have shown that the tendency to segregate through sifting decreases substantially as particle sizes are reduced below 500 microns.^[6] SAFC Biosciences monitors particle size results to ensure the product stays well below the sifting segregation high risk zone.



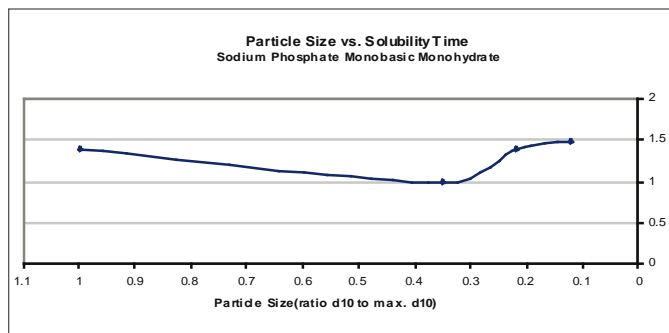
Segregation patterns due to different mechanisms from Jenike & Johanson^[4]

Another common type of segregation occurs through entrainment of air. Since finer particles tend to have lower permeability than coarse particles they generally retain air longer in their void spaces. This results in the coarse particles settling beneath the fluidized fine particles. This is called fluidization segregation and is commonly seen with fine particles. Particles less than 50 μm are also susceptible to entrainment in an air stream, or dusting.^[6] The finer particles remain suspended in air streams longer and can be scattered away to specific points by secondary air currents. A single particle size setpoint will be ineffective in dealing with segregation as both large and small particles have certain tendencies to different types of segregation.

Solubility

Solubilization of the final product is also dependent on particle size. The smaller the particle diameter in a given sample, the larger the total available surface area for the solvent to act. The more surface area available, the faster the rate of solution. In practical applications this does have a limit. When the particle size is reduced too far, the media can agglomerate when the solvent is added. Agglomeration increases the particle size, therefore decreasing the total surface area for the sample and reducing the rate of solution.

SAFC has performed studies on individual components propensity to agglomerate at fine particle sizes. Dissolving 100 grams of Sodium Phosphate Monobasic Monohydrate in 1 Liter of 20°C stirred water at different particle sizes to determine time to solubility (solubility being defined as a



lack of visible particles and a turbidity of <0.85 NTU). Fine particle sizes lead to product agglomeration which increased the overall solubility time.

The critical aspects of media are dependent on particle size but rely on many additional factors as well, such as particle shape and density. These attributes are component-driven and are intrinsic to each recipe. As such, the most advantageous particle size will depend on the recipe, and the unique properties the milled material possesses. SAFC Biosciences hones the controllable variables to deliver a product that preserves flowability, limits the tendency to segregate and maintains solubility.

Future Applications for Powder Characterization

In the future, expect to see further advances in testing technologies that will allow for process design controls to further improve product quality. These developments may include:

- In-line, non-destructive particle size analyzers being used for real time mill controls
- Increased understanding of shear cell testing of samples for a more scientific approach to determining material handling properties
- Improved modeling for greater understanding of complex comminution behaviors

Refining these processes will depend on a fuller understanding of the immensely complex world of defining powder product characteristics. The sheer number of variables and parameters make this a daunting task.

Conclusion

SAFC Biosciences will continue to use the most up-to-date methods for product characterization. The data that particle size analysis provides is an important tool for defining a product. However, the results from particle size testing should be used judiciously, and only for adding value to the process.

Particle size information is a distribution and not a setpoint. Imposing an artificial high limit specification would shift the particle size down. This can lead to creation of a product that is at high risk for certain types of segregation, increased temperatures during milling and may have poor solubility due to agglomeration. Additionally, the material would be difficult to handle and dusty. SAFC Biosciences uses the particle size information as well as many other powder characteristic tests to identify and optimize the properties of each unique product manufactured.

References

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About the Author

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