Particle Technology Labs

PARTICLE DISPERSION FOR SIZE ANALYSIS

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KEY POINTS

The following key points are discussed:

- While particle size analysis can provide great insight in the pharmaceutical industry, it can be nearly meaningless if obtained improperly.

- A particle size distribution can be found for both colloidal dispersions (containing particles <1 µm) and suspensions (containing particles >1 µm).

- A well-dispersed colloid or suspension is one in which the minimum particle size has been achieved and operationally defined by the observance of a constant (minimum) particle size distribution during the dispersion process.

- All sample preparation should begin with microscopic observation of dry powders and liquids "as-is" before dispersion occurs.

- A primary particle is the smallest identifiable subdivision in a particulate system. Primary particles may join to form "soft" (easy to disperse) or "hard" (fused) agglomerates.

- A preparation for liquid particle size analysis is comprised of the material of interest, a carrier, and a surfactant or dispersing agent if needed.

- Selection of a carrier should be based on the chemical composition of the material being tested. Upon dispersion, the material should not dissolve, shrink, swell, or react with the surrounding media.

- Surfactants are amphiphilic materials that reduce the surface tension between the material and carrier, thereby improving the carrier's ability to wet the material if needed.

- If mechanical stirring is not enough energy to separate soft agglomerates, ultrasonic energy may be applied via indirect or direct methods.

- It is important to understand the reasons why determination of the particle size is necessary and how it will be used in a particular process.

- An ultrasonic time study can be useful in determining sample preparation parameters when soft agglomerates are present and when they do not reflect the primary objective of the particle size analysis.

- Dispersed samples must remain stable during the course of analysis.

- A dispersing agent is a substance, that when present in small amounts, improves the kinetic stability of particles and improves dispersion.

INTRODUCTION

Particle size analysis is a vital analytical tool in the pharmaceutical industry. The results can be used to meet safety and quality specifications and provide information for product development and manufacturing. Modern techniques have made determining the particle size of a material quick and convenient, but many factors can influence the quality of data obtained.

While particle size analysis can provide great insight, it can be nearly meaningless if it is obtained improperly. A large number of resources are available from notable institutions such as the International Organization for Standardization (ISO), United States Pharmacopeia (USP), and American Society for Testing and Materials (ASTM). These organizations provide standardized literature to help reduce common sources of error, improve accuracy, and increase reproducibility using various particle-sizing techniques. Errors observed in the generation of particle size data occur due to the selection of a technique inappropriate to the material type or through the use of poor sampling and dispersion techniques (i.e., sample preparation). In many cases, the end application in which the particle size will be used is not properly considered, thus the amount of dispersion needed is inadequate or overcompensated. While each source of error is equally important to address, only the aspects pertaining to achieving a well-dispersed system for solid in liquid particle size analysis are covered in this discussion.

DEFINING DISPERSION TERMINOLOGY

A dispersion is defined as "a two-phase system in which discontinuities of any kind (solid, liquid, gas) are dispersed in a continuous phase of a different composition or state" (1). For the purposes of this article, this definition will pertain to solids within a liquid but can just as easily apply to particle size analyses performed on emulsions (i.e., liquid in liquid) or dry powders (i.e., solids in gas). Modern sizing techniques generally focus on two types of dispersions (colloidal dispersions and suspensions) that are defined by specific properties and their response to gravity. They are defined as follows (2):

- Colloidal dispersions

- Contain particles < 1µm in size.

- Particles exhibit random movement due to collisions with the molecules of the surrounding media (Brownian motion).

- Do not settle over time due to the effects of gravitational forces when stable.

- Particles in the system scatter light equal to or less than their size. Colloidal particles thus predominately scatter blue light as exhibited in the Tyndall effect.

- Particles have a large surface area to volume ratio that greatly affects the interactions at the solid and liquid interface dictating the stability of the dispersion. Example: paint.

- Suspensions

- Contain particles> 1μm in size.

- Particles settle due to the effects of gravity.

- Particles in a suspension scatter more of the visual spectrum of light (not only blue but red light as well).

- Interactions at the solid and liquid interface play a minimal role in particle dispersion behavior in comparison to the effect of gravity. Example: sand in water.

A solution is not considered a colloid or a suspension. The term solution is used to describe a mixture of two or more components that are homogenous on a molecular level (i.e., < 1 angstrom) characterized by a clear or transparent appearance that cannot be separated into pure components (2).

Most instruments and techniques in use today (e.g., laser diffraction, light obscuration, and dynamic light scattering instruments) cannot distinguish two particles attached to one another from one single large particle due to inadequate dispersing techniques. Thus when two attached particles (also called a doublet) are measured, the instrument reads them as a single larger particle (3). Measurements taken when particles are poorly dispersed can lead to results that may change in size over the course of the analysis (producing variability) or fail to represent the actual primary size of the material. It is, therefore, important to identify the particles' state of agglomeration and the best method of dispersion. The sample for analysis must be well dispersed, stable, and representative of its true size throughout the analysis. In short, a well-dispersed system is described as follows:

"A stable suspension (or colloid) in which the minimum particle size has been achieved and operationally defined by the observance of a constant (minimum) particle size distribution during the dispersion process" (1).

OBSERVING PARTICLES WITHIN A MATERIAL

Observations made by microscopy of the material during particle size method development can play an important role. For material typically larger than a few microns, a standard light or polarized microscope can be utilized to make these observations. However, many sample types have optical properties that make viewing and obtaining information with a common light microscope quite difficult.

Minerals, chemical crystals, colloidal particles, materials with refractive indices close to the medium's in which they are suspended, or materials with textured surfaces benefit immensely from a technique known as dark-field illumination. This style of microscopy allows only scattered light to pass through to the viewing objective so the object in view appears bright on a dark background. The image on the left side of Figure 1 shows crystals illuminated with a traditional light microscope. It is only when the material is illuminated using the dark-field configuration (Figure 1, right) that the facets and irregularities of the larger crystals become more defined (4). In many cases, dark-field can also enhance the evaluation of the fines (small particles) population. Observing sample preparations by microscopy provides the analyst guidance and a mode of comparison in assessing the validity of the particle size distribution obtained by instrumentation. Microscopic observations also provide valuable insight into the particle shape, which is another important consideration when evaluating particle size results. For material that is smaller than a few microns, the use of an electron microscope is recommended where applicable.
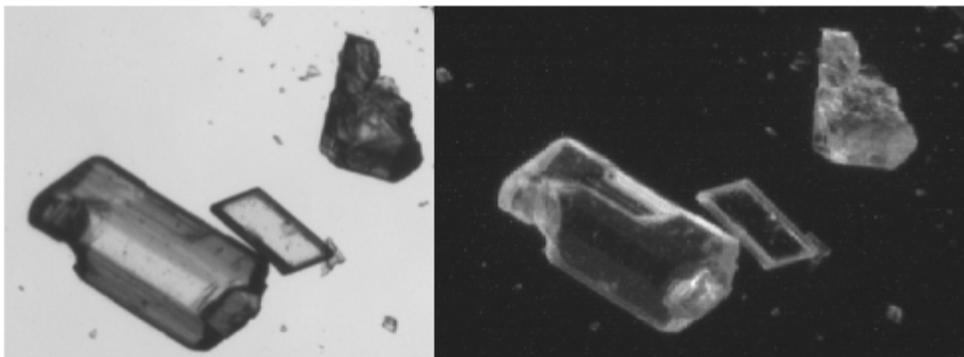
FIGURE 1. Material under light microscopy (left) and dark-field microscopy (right).

For best practices, all sample preparation should begin with microscopic observation of dry powders and liquids "as-is" before dispersion occurs. A small representative portion of the sample should be placed upon the microscope slide, and its dispersive qualities should be reported. A short list of characteristics to note could entail the following:

• Dry powder samples

• General size of primary particles

• Shape and color of particles

• Type(s) of agglomerates present

• Powder qualities (sticky or free-flowing)

• Liquid dispersion samples

• General size of primary particles

• Shape and color of particles

• Type(s) of agglomerates present

• Concentration of particles (presence of multiple populations and relative amounts compared to each other)

• Stability of the system.

At this point, concerns about whether the sample type, given its noted characteristics, is appropriate for the selected analytical technique should be resolved. If the primary size or shape of the material is not within the recommendations of the selected particle sizing technique, an alternative one must be chosen. In addition, review of the desired data output formatting (i.e., volume or number basis) should occur to determine if it is applicable to the task at hand. Many manufacturers produce instruments that collect and present data using a primary calculation (e.g., volume) and give the option to convert to other presentations (e.g., number) or vice versa. However, these conversions are done based on secondary calculations and assumptions. As a result, the data should be viewed with caution for non-true Gaussian (or monomodal) distributions.

UNDERSTANDING PRIMARY PARTICLES AND AGGLOMERATES

The National Institute of Standards and Technology defines a primary particle to be the smallest identifiable subdivision in a particulate system (1). These particles may be well-formed crystals or irregular-shaped fragments. Under certain conditions, these primary particles may join and fuse together to form assemblages (or agglomerates) of primary particles. Milling, micronizing, and certain storage conditions can cause particles to be more likely to absorb small amounts of water, which can cause partial dissolution at the Particle surface. Particles undergoing re-

crystallization that are in contact with one another can fuse together, forming what are known as hard agglomerates (5). These agglomerates are made of several individual particles, but because they are fused, they cannot be easily separated without fracturing the newly formed particle assemblage At this point, it is important to consider if what is being observed microscopically is reflective of dispersed particles or agglomerates and whether the particles are representative of the sample as used in the current processing environment or the research question being investigated. If the hard agglomerates are representative of the current process, care should be taken when applying additional dispersive energy to the sample to avoid destroying these complexes.

Figure 2 shows an example of a powder adequately dispersed in a liquid that contains hard agglomerates composed of fused primary particles. This is in contrast with soft agglomerates that are not fused together as steadfastly and typically disperse easily with minimal energy (e.g., mixing, sonication, vortexing, etc.) or with the application of surfactants. Note that the necessary amount of energy needed to disperse soft agglomerates will not only be determined by the material's physical and chemical properties, but also by those of the carrier and dispersing agents.
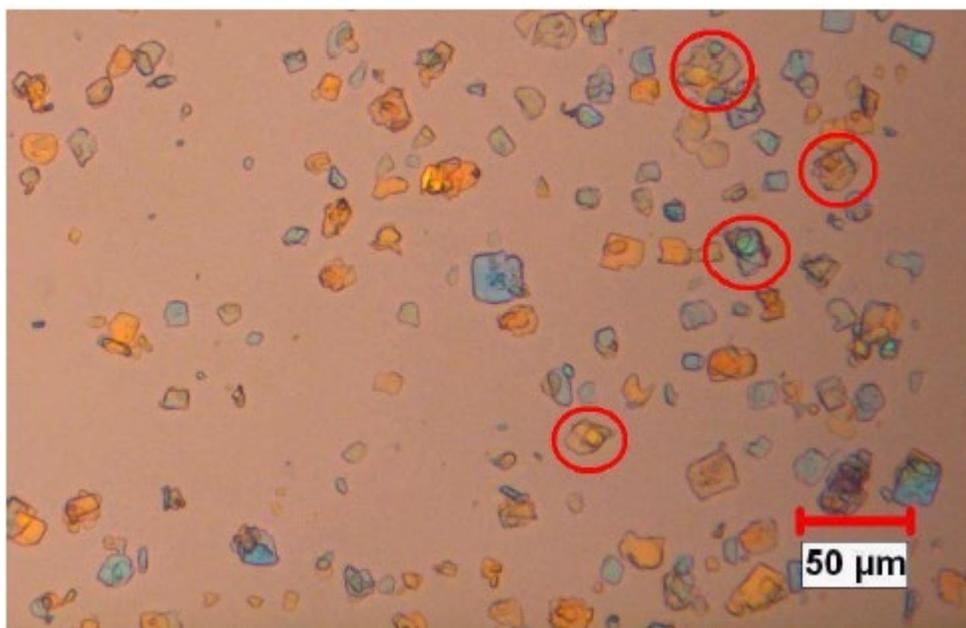

FIGURE 2. An example of hard agglomerates (fused primary particles) ringed in red.

As noted previously, the reason a complete dispersion of primary particles is needed is because particle size analyzers cannot determine the difference between a group of soft agglomerates composed of several primary particles and one large primary particle by itself. In both cases the instrument will register each one as one particle, but possibly of a different size. This size difference can lead to a misconception about the actual size of the material if microscopic observations have not been performed (3).

Particle size analysis with the proper dispersion technique can provide a substantial amount of information in regards to what happens to a material during the course of a product's life cycle. For example, perhaps a stability study is warranted on a freshly micronized pharmaceutical product over the course of several time points and storage conditions. In this scenario, there is a need to monitor agglomeration during the study. The data collected at T=0 months cannot be accurately compared to T=24 months if the dispersion created at the beginning of the study was poor and not representative of the material or the primary particle size. In addition, care must be taken in how many dispersion mechanisms are utilized at each time point as formingagglomerates could be artificially dispersed (i.e., the material may be increasingly agglomerated but more aggressive dispersion techniques have created an erroneous false negative).

PREPARING DISPERSIONS—CARRIER SELECTION

ISO 14887: Sample Preparation-Dispersing Procedures for Powders in Liquids is one of several useful resources for preparing dispersion samples using particle size analyzers (6, 7). When placing a material into a carrier, intermolecular interactions are occurring at the liquid/solid surface interface (8). It is best to refer to a collective dispersion (i.e., carrier, material, and surfactant or dispersing agent if needed) as a "system." Adjusting any variable pertaining to the system may help or hinder the dispersion and its applicability for particle size analysis. The effectiveness of adjusting the system parameters will depend on the particle size of the material (i.e., colloid vs. suspensions).

Selection of a carrier should be based on the chemical composition of the material being tested. The material should not dissolve, shrink, swell, or react (3) within the suspension media. If the material is currently in a liquid, dilution may be necessary to reach a specified concentration range given by the instrument in use. Calculation errors, such as multi-scattering (i.e., light scatter from multiple particles instead of from a single particle due to high concentration) or coincidence counting in the case of stream counters, can be minimized with further dilution of the sample.

This being said, care must be taken to avoid drastically changing the dynamics of the system to avoid destabilizing it. This is especially true for injectables, oral suspensions, and products that have been formulated for stability in a particular liquid suspension environment. Under these conditions, it may be necessary to either use a "mother liquor" (i.e., the solution containing the suspended particles obtained after vacuum filtration or centrifugation) or replicate the liquid media component of the system in such variables as pH, conductivity, and chemical composition to keep the system stable when dilution is necessary. Dilution using the same ionic environment is of the utmost importance when working with colloidal dispersions as the particle's surface area/volume ratio is greatly increased in these systems.

A selected carrier should also be able to wet the material (i.e., thoroughly spread out when deposited onto the solid) (9). If the material does not wet easily when placed into a selected carrier, the surface energy, or the energy that must be supplied to increase the surface area by one unit at the interface between the particle and the liquid, may be very high. This energy can be calculated through contact angle measurement (i.e., the angle at which a liquid/vapor interface meets the solid surface) (10). The relationship between the interfacial energies involved can be summarized by Young's equation, which is as follows:

$$O = \gamma SG - \gamma SL - \gamma LG \cos\theta$$

Where γSG, γSL and γLG are the interfacial energy at the solid/gas, solid/liquid, and liquid/gas interface.

If the surface energies are low between the solid and the liquid, it will completely spread out on the solid surface, and the contact angle between the two will be very small (approaching 0°). This is common for water on a hydrophilic solid, as illustrated in Figure 3. Hydrophobic materials placed into water generally develop a contact angle that is very large (greater than 90°) (10). This can cause the material to float on the carrier surface or lessen the effectiveness of sonication when applied to the suspension.
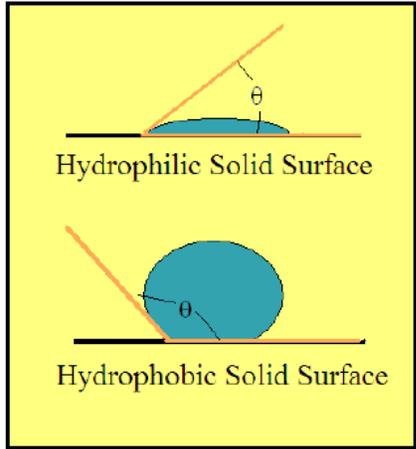
FIGURE 3. Contact angle values for hydrophilic and hydrophobic materials.

A comparison of wettability is illustrated in Figure 4, which demonstrates the dispersions created when magnesium stearate is placed in water and in 2-propanol. Because magnesium stearate is hydrophobic, placing it in water causes it to remain on the surface in stead of breaking the surface tension of the carrier. This is due to the greater contact angle between the liquid carrier and the material. However, when magnesium stearate is placed in 2-propanol, the material disperses easily as seen by formation of the cloudy suspension in the carrier by simply swirling the flask.

FIGURE 4. Dispersion of magnesium stearate into 2-propanol (left) and water (right).

THE APPLICATION OF SURFACTANTS

According to Mueller (11), surfactants (also known as surface active materials) are substances that decrease the surface energy. These surface active materials are amphiphilic, or possess both hydrophobic and hydrophilic properties. They align themselves at the boundaries between solid and liquid to lower interfacial tension and improve wetting of the material. An example of a surfactant used to improve wetting in aqueous systems would be Polysorbate 80 or Triton X-100. When adding a surfactant to a carrier to use during analysis, the chemical properties and concentration of the selected surfactant must be considered. Because surfactant molecules contain both hydrophobic and hydrophilic regions, they have the ability to aggregate into "colloidal-size" clusters known as micelles (see Figure 5). These form under the right conditions when the amount of surfactant in an aqueous carrier reaches a specific concentration, known as the critical micelle concentration (CMC) (12).
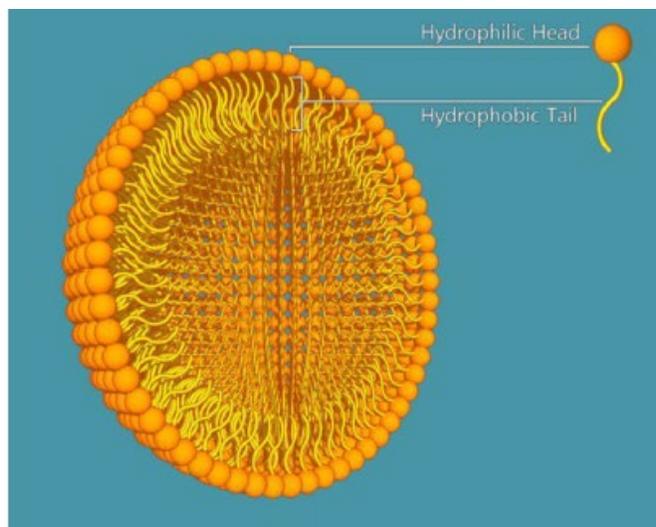
Image courtesy of Brandon Sharas (2011)

FIGURE 5. Surfactant molecule and the cross-sectional view of a micelle in an aqueous carrier

It is important to keep the amount of surfactant below the CMC to prevent micelles from forming where they themselves may be measured, producing biased results. Certain instruments, such as dynamic light scattering instruments, have the capability of detecting particles the size of micelles and can not differentiate between the particles of interest and the micelles (3).

DISPERSING AGGLOMERATED MATERIAL

Once a suitable carrier and surfactant (if required) has been found, it is important to reassess the sample by microscopy once it has been dispersed. An analyst should then ask the following questions:

- Have the initial soft agglomerates seen by microscopy dispersed?

- Is further dispersion required to obtain the primary particles size?

- Does the sample preparation appear stable (i.e., do the particles appear free-flowing and resistant to congregating into flocs)?

If soft agglomerates are still present, the application of additional dispersive energy to the system may be helpful. This can be done by low energy agitation (e.g., hand mixing, overhead stirrers, or vortexers) or by higher energy agitation such as ultrasonic baths and probes. The low energy types of dispersive agitation generally work well for large particles but may have little effect on smaller particles. This is due primarily to the cohesive forces at work between particles, which increases as particle size decreases (5). High attractive forces between small particles (e.g., 10 pm) is demonstrative of the fact that the same test method developed for un-micronized material cannot typically be used when analyzing micronized material. The micronized material requires additional dispersive energy to overcome the particle/particle attractive forces.

USING ULTRASONIC ENERGY TO DETERMINE PRIMARY PARTICLE SIZE

If mechanical stirring is not enough energy to separate soft agglomerates, the use of ultrasonic energy (UL) may be warranted. Ultrasonic energy uses sonic waves roughly at 20 kHz and greater (13) and produces alternating high and low pressure cycles in which vacuum bubbles form and implode. These impulses release localized energy and heat that help to disperse and deagglomerate particles (14). While there are many types of ultrasonic equipment available, it is necessary to determine the most appropriate for the sample—one that generates the primary particle size dispersion without causing fragmentation, breakage, fracturing, or chemical reactions of the particles due to energy released.

Sonication may be applied indirectly or directly to a sample preparation. Indirect sonication does not allow the prepared material to come in contact directly with the ultrasonic energy. For example, in an ultrasonic bath, the ultrasonic waves must move through the liquid in the bath and then through the wall of the container holding the prepared sample (15). This technique works well for large or more fragile or friable material.

Using direct sonication, ultrasonic energy is directly introduced to the sample by use of an ultrasonic probe. This will apply a higher ultrasonic intensity to the sample in comparison to using a bath. Direct sonication may work well for robust, sub-micron material, such as zircona and titania. However, prolonged sonication may increase the temperature of the preparation potentially causing physical or chemical changes to sensitive materials. Precautions should be taken to reduce the likelihood of this occurring (16).

Ultrasonic Time Studies

An ultrasonic time study, in which a change in particle size is observed with respect to ultrasonication time, should be performed in conjunction with microscopic observations to ensure the results obtained using a particle size analyzer is reflective of the primary particles size. This information supports reproducibility during method development and ensures that the preparation being tested provides consistent results between analysts and lots. A sample dispersion analyzed with agglomerates can misrepresent the primary particle size or produce variable results during the analysis.

An ultrasonic time study is presented in the Table and Figure 6. A drug product was prepared in an organic carrier and analyzed by a laser diffractor. After hand-mixing and then applying ultrasonic energy for three specific lengths of time using an ultrasonic bath, the average particle size results (n 4) were reported.

Table 1.
Average Laser Diffraction Particle Size Data Summary
Ultrasonic Time Study

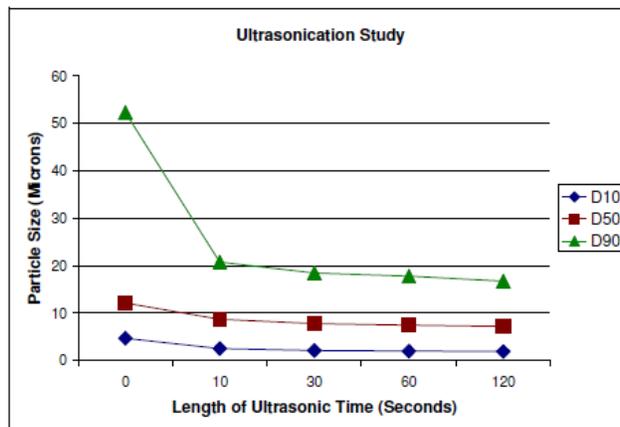| Applied UL	Cumulative Volume % Less Than Indicated Size		
	Dv10	Dv50	Dv90
0 Seconds	4.72	12.17	52.29
10 Seconds	2.54	8.7	20.77
30 Seconds	2.14	7.81	18.46
60 Seconds	2.00	7.49	17.76
120 Seconds	1.89	7.16	16.75

FIGURE 6. Ultrasonic time study based on the Table.

The results obtained after immediately dispersing the particles in the carrier without sonication (using only hand mixing/shaking) shows 90% of the material (Dv90) is less than 53 microns. A photo microscopy image (Figure 7) represents the hand-mixed sample and clearly shows a poorly dispersed preparation containing very large soft agglomerates greater than 100 microns in diameter. Evaluation of the individual measurements of this initial time point found a decreasing particle size during analysis as the loosely held soft agglomerates were dispersing due to the applied shearing forces imparted by the instruments recirculation device. Ideally, no change over the course of several measurements or loops on the sample aliquot should be seen.
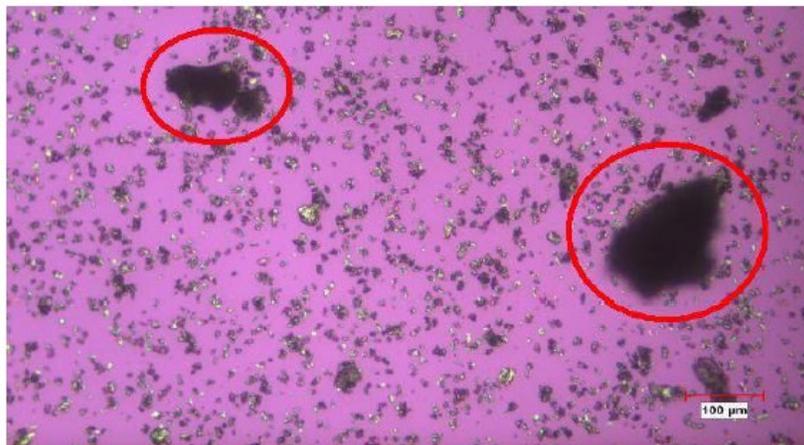

FIGURE 7. Soft agglomerates ringed in red present in the hand-mixed dispersion.

Introducing ultrasonic energy caused a significant and swift decrease in the particle size over the next specified time points as the agglomerates were dispersed. The particle size distribution was stable during the course of the four measurements at each ultrasonic time point. The photo representing the prepared material after 60 seconds of sonication (Figure 8) shows an absence of soft agglomerates and is more representative of a stable, well-dispersed system. At first glance, this would be a suitable place to conclude further application of ultrasonic energy. Further statistical analysis using factorial design would be able to confirm or predict if additional sonication time is needed.
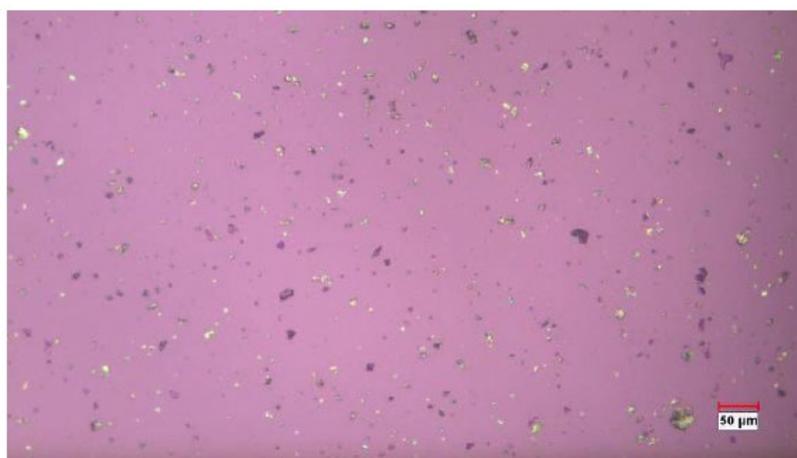

FIGURE 8. Prepared sample analyzed after 60 seconds of UL energy.

From a quality assurance viewpoint, it is important to record the parameters used in creating a good dispersion through the use of ultrasonication. These documentation practices should be well established in GXP environments and will make the validation or transfer of a developed method much more manageable. Some of the parameters that are necessary to document in a particle size test method include the duration of ultrasonic energy, type of ultrasonic instrument (bath or probe), and properties of the device used (bath volume dimensions or horn size).

The concentration of the prepared material and power output of the ultrasonic device are factors that can influence the effectiveness of the generated cavitation (16). While adjusting the dilution ratio is an easy problem to rectify, the "power" of the ultrasonic device can be difficult to quantify and document. The energy applied to the sample dispersion is related to numerous instrument and mechanical factors that must be considered—what works in one lab may not work in another due to equipment differences. In many cases, documentation of the device used and good laboratory practice (e.g., ensuring clean ultrasonic bath liquid at the proper operational level) coupled with microscopy observations of the dispersed sample will allow for comparable results between labs. If primary particle size results are the goal, adjustment of ultrasonication time or type may be required between laboratories to account for differences in equipment.

STABILIZING THE DISPERSION

Once the particles have been initially dispersed, it is important that the entire system remain stable during the course of analysis. A system that contains particles that slowly floc into loosely clustered assemblages is not considered stable and will likely cause significant variability in the particle size data. Figure 9 shows the same material in an unstable system vs. a properly dispersed stable system.

FIGURE 9. Flocs emphasized by the red rings in an unstable dispersion (left) which are not present in a stable dispersion (right)

When a material is placed into a carrier, activity at the liquid/solid interface can cause particles to acquire a charge. For example, a particle placed in an aqueous solution can possess functional groups on the solid surface that give the particle a positive charge. In order to diffuse this charge, an electrical double layer is formed around the particle composed of ions in the carrier. In the particular example above, negatively charged ions in contact with the surface make up a region called the Stern layer. As the distance increases from the particle's surface, additional ions (both positive and negative) gather due to their attraction to the positive particle and negatively charged ions within the Stern layer. This additional layer further neutralizes the system electrically and is known as the diffuse layer.

It is the electrical attractive and repulsive forces within the layers mentioned above at both a molecular level and at a particle level that dictate whether the strength and size of the double layer will repel two particles and keep them apart or attract and cause flocculation and eventual sedimentation. Colloidal dispersions are especially susceptible to these forces acting at the solid/liquid interface due to their high surface area to volume ratio and the strong influence on particles due to Brownian motion. Particles greater than 1 µm in size (depending on their density) will most often be more affected by gravitational forces due to the Navier Stokes Law. The electrical repulsive or attractive forces governing dispersion play a much smaller role in

these systems. This stresses the development of a unique method to properly disperse a product during different stages of production where the effects of these forces are more prominent (as seen in micronized material in comparison to unmilled material). Figure 10 shows a simple drawing of the electrical double layer around a particle. A common way of gaining insight into the stability of a colloid in an aqueous medium is to determine the zeta potential (i.e., the electrical potential at the point between the stern layer and the diffuse layer) of the system (8).
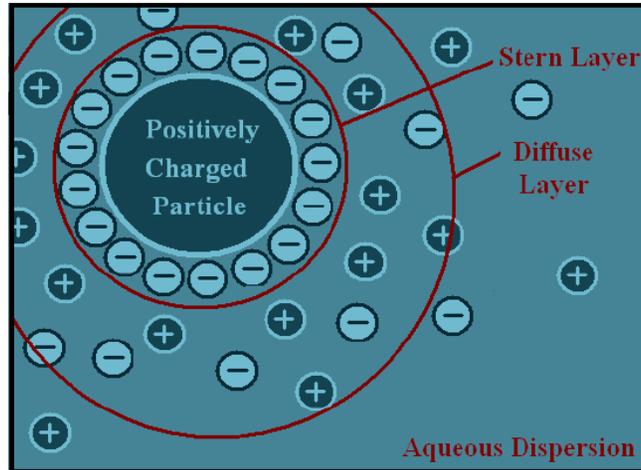
FIGURE 10. The electrical double layer.

While this is a highly complex topic, it is important to understand that these electrical forces can be manipulated into intentionally creating stable (or unstable) dispersions by adjusting the environment around the particle. The most common ways of doing this are through electrostatic and steric stabilization.

Electrostatic, Steric, and Electrosteric Stabilization

Electrostatic stabilization involves changing a variable within a system such as pH or ionic concentration to cause a change to occur in the electrical double layer, thus resulting in greater stability or instability. The use of a dispersing agent (i.e., a substance that, when present in small amounts, improves the kinetic stability of particles) (1) can be instrumental in obtaining a good dispersion. Common examples include sodium pyrophosphate and Daxad 19. It is critical to consider the chemistry of the liquid component when diluting for particle size analyses in order to avoid negatively affecting the stability of the system (8).

Electrostatic stabilization may not be a viable option in certain samples (e.g., if the conditions surrounding the particles to be analyzed must not be altered in order to prevent changing how a product is normally used eg., drugs made for intravenous use, or if the material must be analyzed in a nonpolar (i.e. hydrocarbon) media due to chemical incompatibility with water). Charge stabilization is less effective in non-aqueous media, and in these situations, steric stabilization may help to improve the dispersion (17).

Steric stabilization involves the addition of long chained polymers that effectively produce a repulsion due to entropic stabilization between like particles. The best steric stabilizers are known to be amphiphilic (i.e., one part of the molecule has an affinity for the dispersion media, while another portion has an affinity for the particle and becomes an anchor to its surface). Electrostatic and steric stabilization can be combined into what is known as electrosteric stabilization. In this situation, the repulsive charge can be caused by the net charge on the particle surface or a charge associated with the polymer anchored to the particle (17).

A stable dispersion allows for the particle size analysis to occur without many of the common problems that plague method development, such as an increase in particle size over time due to the flocculation and eventual sedimentation of particles. Without stability, or any other element of a well-dispersed preparation, the repeatability of the method suffers, resulting in a less than ideal method. These results cannot be used as a basis for comparison when conditions in the production process are changed nor can they be relied upon for effective quality control.

CONCLUSIONS

Particle size analysis is a vital analytical tool in the pharmaceutical industry. While particle size analysis data can provide great insight, it can be nearly meaningless if it is obtained improperly. An invaluable element of obtaining reproducible and representative particle size results is the development and maintenance of a well-dispersed system. This includes using observations made by microscopy in combination with chemical knowledge of the material in choosing appropriate carriers and dispersing agents to produce a stable dispersion. Once achieved, the analyst can then decide, based on particle characteristics, if additional dispersive energy is needed to acquire results most representative of the product. Methods developed with these variables in mind allow for enhanced quality control and a better understanding of the product.

REFERENCES

1. Hackley, Vincent A., and Chiara F. Ferraris, The Use of Nomenclature in Dispersion Science and Technology, Washington: US Government Printing Office, Special Publication 960-3, 2001.

2. Bettelheim, Frederick A., and Frederick A. Bettelheim, Introduction to General, Organic, and

Biochemistry, Belmont, CA: Thomson, Brooks/Cole, 2007.

3. ISO, "Sample Preparation—Dispersing Procedures for Powders in Liquids," International Standards Organization ISO 1488 7:2000.

4. Abramowitz, Mortimer, and Michael W. Davidson, "Molecular Expressions Microscopy Primer: Specialized Microscopy Techniques-Darkfield Illumination," Molecular Expressions: Images from the Microscope, Jan. 4, 2011. <http://micro.magnet.fsu.edu/primer/techniques/darkfield.html>.

5. Nichols, Gary, MarkJ. Bloxham, Joanne Botterill, Neil J. Dawson, Andrew Dennis, Valerie Diart, Nigel C. North, and John D. Sherwood, "A Review of the Terms Agglomerate and Aggregate with a Recommendation for Nomenclature Used in Powder and Particle Characterization, "Journal of Pharmaceutical Sciences 91: 2103-109, 2002.

6. ISO, "Particle Size Analysis—Laser Diffraction Methods," International Standards Organization ISO 13320:2009.

7. ISO, "Particle Size Analysis—Laser Dynamic Light Scattering (DLS)." International Standards Organization ISO 22412:2008.

8. Gregory, J., Particles in Water Properties and Processes, London: IWA Pub., 2005.

9. Brochard-Wyart, Francoise, David Quere, and Pierre-Gilles De.Gennes, Drops, Bubbles, Pearls, Waves: the World of Capillarity, New York: Springer, 2003.

10. Förch, Renate, Surface Design: Applications in Bioscience and Nanotechnology, Weinheim: Wiley-VCH-Verl., 2009.

11. Müller, Bodo, and Ulrich Poth, Coatings Formulation: an International Textbook, Hannover: Vincentz, 2006.

12. Rosen, Milton J., Surfactants and Interfacial Phenomena, Hoboken, NJ: Wiley-Interscience, 2004.

13. Cheeke,J. David N., Fundamentals and Applications of Ultrasonic Waves, Boca Raton: CRC, 2002.

14. Hielscher, Thomas, Ultrasonic Production of Nano-Size Dispersions and Emulsions. Proc. of European Nano Systems, Paris, France, 2005.

15. Capelo-Martinez,Jose-Luis, Ultrasound in Chemistry: Analytical Applications, Weinheim: Wiley-VCH, 2009.

16. Jillavenkatesa, Ajit, StanleyJ. Dapkunas, and Lin-Sien H. Lum, Particle Size Characterization, Washington: US Government Printing Office, 2001.

17. Shi, Jingyu, Center for Industrial Sensors and Measurements, Department Materials Science & Engineering Group, Inorganic Materials Science, Literature Review: Steric Stabilization. Materials Science and Engineering Department. Ohio State University, August 29, 2002. http:llwww.matsceng.ohio-state.edu/ims/LR_Electrosteric.pdf.

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